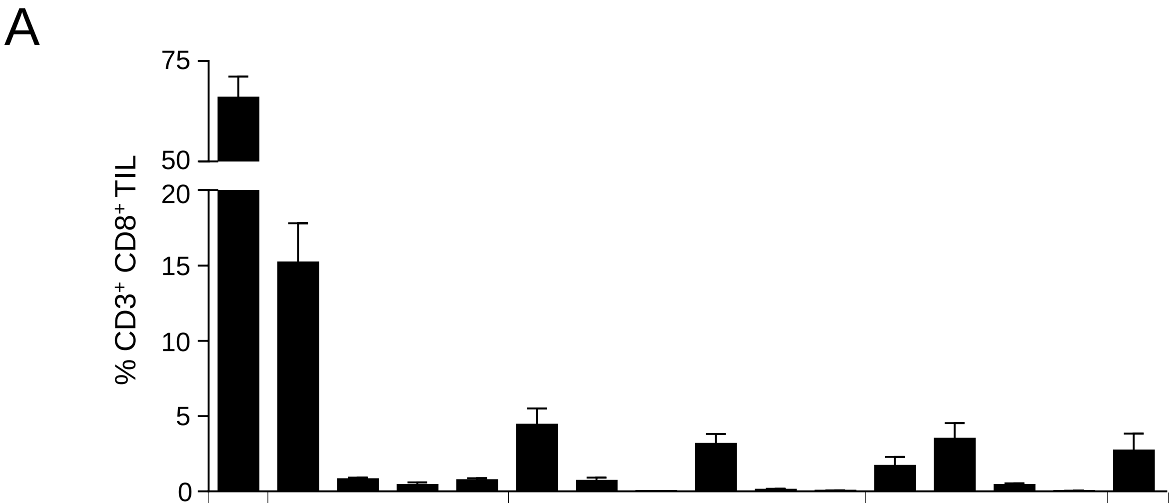
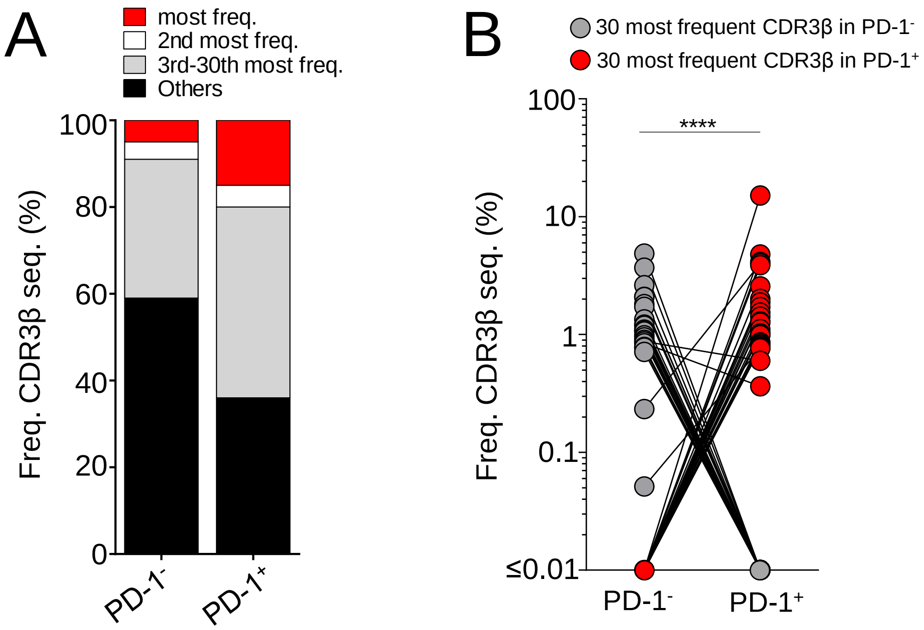
Supplementary Figure 1



PD-1	-	+	-	-	-	+	+	-	+	-	-	+	+	+	-	+
4-1BB	-	-	+	-	-	-	-	-	+	+	+	-	+	+	+	+
LAG-3	-	-	-	+	-	-	+	+	-	-	+	+	-	+	+	+
TIM-3	-	-	-	-	+	+	-	+	-	+	-	+	+	-	+	+

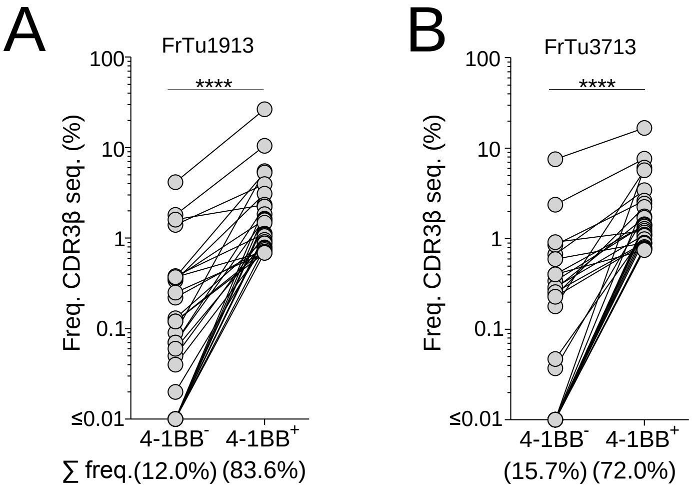
Supplementary Figure 1. Boolean gate analysis to study co-expression pattern of PD-1, LAG-3, TIM-3 and 4-1BB in CD8$^+$ TIL. All possible permutations of expression of PD-1, LAG-3, TIM-3 and 4-1BB on CD8$^+$ TIL were analyzed with boolean gate analysis. Expression of individual or combination of markers is shown as the mean±s.e.m.

Supplementary Figure 2



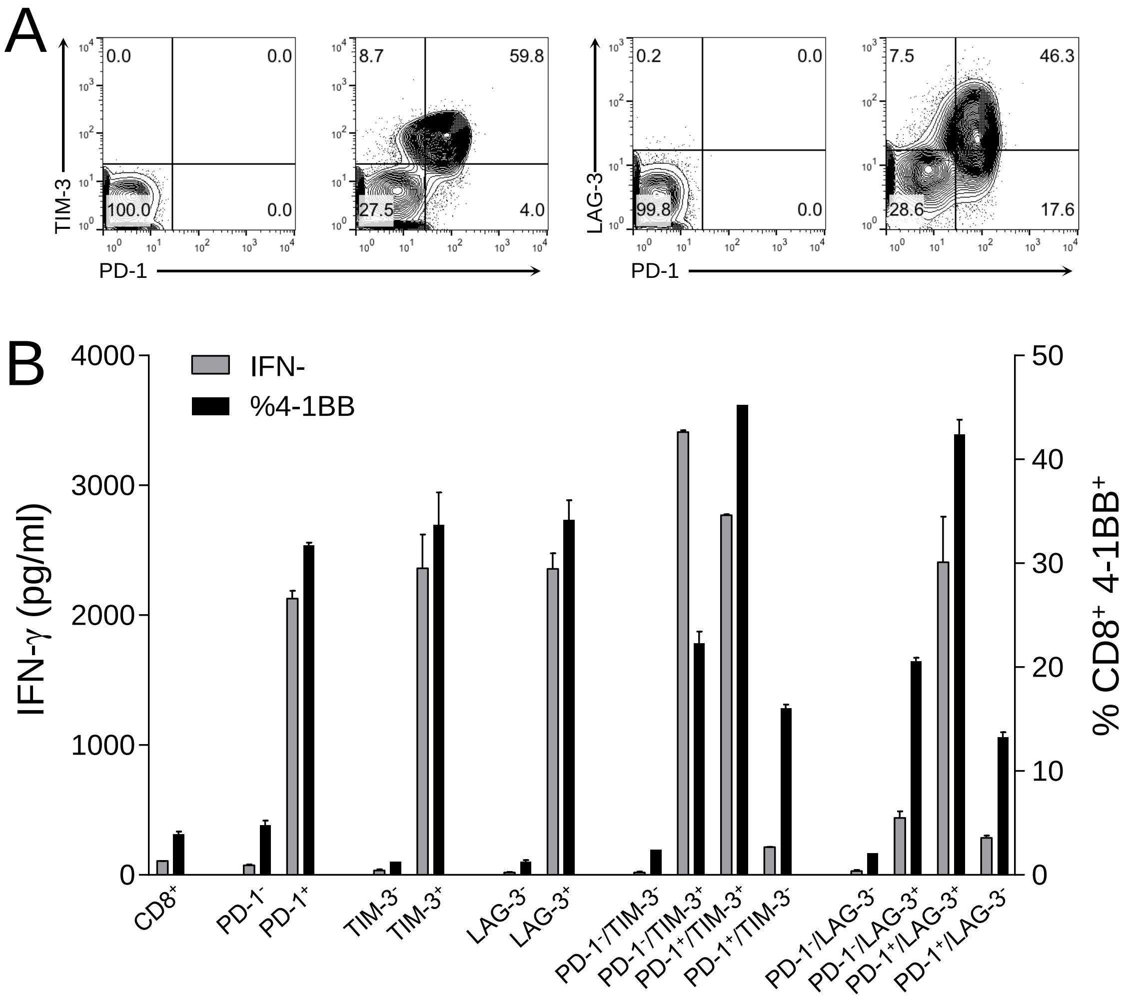
Supplementary Figure 2. TCRβ deep sequencing of CD8^{+} TIL from FrTu3612. CD8^{+}PD-1^{+} and PD-1^{-} TIL were sorted to high purity from fresh melanoma digest FrTu3612, mRNA was extracted, and TCRβ deep sequencing was performed. (**A**) Diversity of the TCRβ repertoire within the CD8^{+}PD-1^{-} and PD-1^{+} populations. The relative frequencies of the most frequent TCRβ clonotype (unique CDR3β amino acid sequences), the second most frequent, the 3rd to 30th most frequent, and the rest of the clonotypes are shown. (**B**) Frequency of the thirty most frequent CD8^{+}PD-1^{+} and PD-1^{-} TCRβ clonotypes. The frequencies of the most represented clonotypes and their frequencies in the PD-1^{+} and PD-1^{-} populations are shown. Each dot represents one unique TCRβ clontoype.*****P*≤0.0001, significant by Wilcoxon signed-rank test.

Supplementary Figure 3



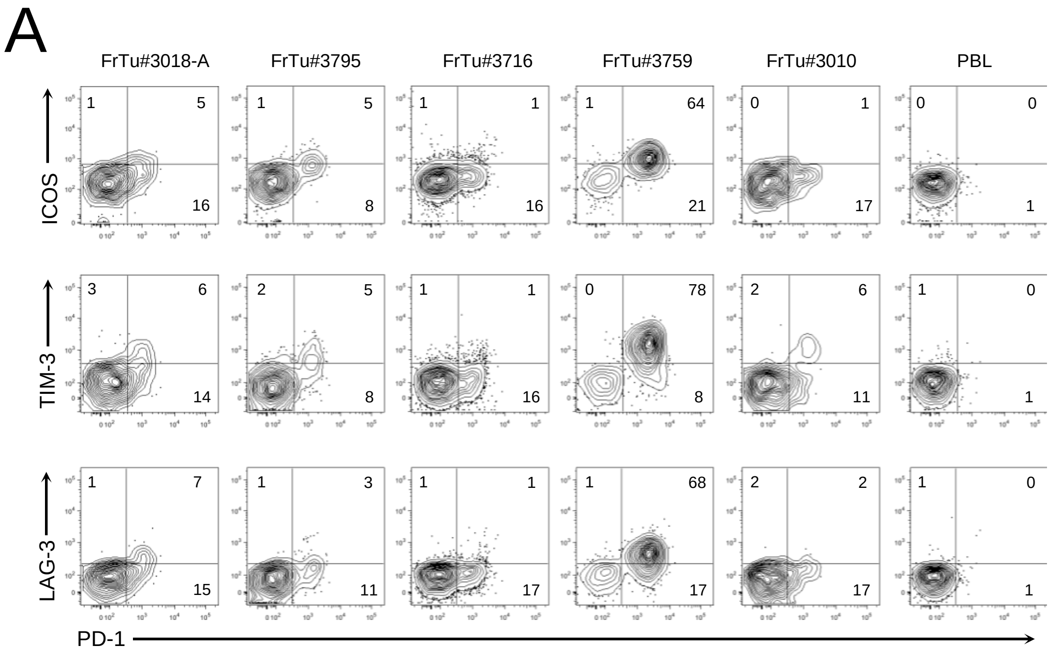
Supplementary Figure 3. TCRβ deep sequencing of CD8$^+$ TIL separated based on expression of 4-1BB in the tumor. Frequency of the thirty most frequent TCRβ clonotypes in the CD8$^+$4-1BB$^+$ population of (**A**) FrTu1913, and (**B**) FrTu3713. The frequencies in the 4-1BB$^+$ and 4-1BB$^-$ populations are shown. The cumulative frequency (∑ freq.) of the clonotypes present in each of the populations is shown below.*****P*≤0.0001, significant by Wilcoxon signed-rank test.

Supplementary Figure 4



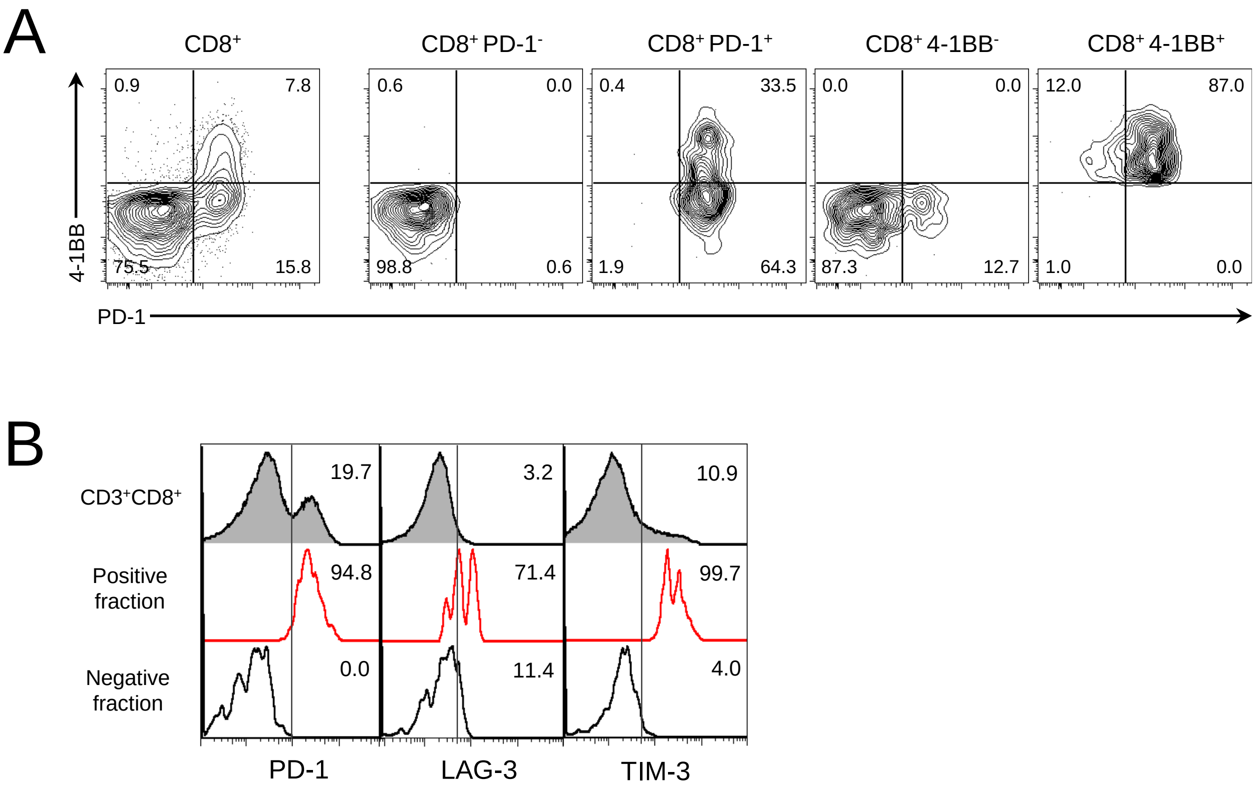
Supplementary Figure 4. Response of CD8$^+$ TIL subpopulations derived from FrTu3289 to autologous tumor cell line TC3289. CD8$^+$ TIL were sorted from FrTu3289 into CD8$^+$, PD-1$^-$, PD-1$^+$, TIM-3$^-$, TIM-3$^+$, LAG-3$^-$, LAG-3$^+$, PD-1$^-$/TIM-3$^-$, PD-1$^-$/TIM-3$^+$, PD-1$^+$/TIM-3$^+$, PD-1$^+$/TIM-3$^-$, PD-1$^-$/LAG-3$^-$, PD-1$^-$/LAG-3$^+$, PD-1$^+$/LAG-3$^+$, and PD-1$^+$/LAG-3$^-$ and expanded in vitro for 15 days with OKT3, IL-2 and irradiated feeders. (**A**) Phenotype of CD8$^+$ TIL in FrTu3289. FrTu3289 was thawed and rested overnight in absence of cytokines. Dot plots show the background staining (isotype control) and expression of TIM-3 by PD-1 or LAG-3 by PD-1. (**B**) Response of CD8$^+$ TIL subpopulations expanded from FrTu3289 to autologous tumor cell line TC3289. The mean IFN-γ release and 4-1BB upregulation (mean±s.d., duplicates) 24h after coculture is plotted.

Supplementary Figure 5



Supplementary Figure 5. PD-1 expression on CD8$^+$ melanoma TIL is associated with ICOS, TIM-3 and LAG-3 expression. (**A**) Phenotypic characterization of tumor-infiltrating CD8$^+$ lymphocytes. Dot plots depict the frequency of expression of PD-1, ICOS, TIM-3 and LAG-3 on tumor-infiltrating and peripheral blood CD8$^+$ lymphocytes (TIL n=5; PBL n=1), and the pattern of co-expression of ICOS, TIM-3 or LAG-3 with PD-1.

Supplementary Figure 6



Supplementary Figure 6. Post-sort purity of cell subsets sorted from fresh melanoma tumors. (**A**) Representative dot plots of CD3$^+$CD8$^+$ TIL sorted from FrTu1913 according to PD-1 and 4-1BB expression used for TCRβ sequencing. The post-sort purity of each of the populations sorted (bulk CD8$^+$TIL, CD8$^+$PD-1$^+$, CD8$^+$PD-1$^-$, CD8$^+$4-1BB$^+$ and CD8$^+$4-1BB$^-$) is shown. (**B**) Representative histograms of FrTu1913 CD3$^+$CD8$^+$ T cells sorted based on expression of PD-1, LAG-3, and TIM-3 expression. The name of the marker used for separation of the cells is specified below each histogram. The frequency of expression of each of the markers in the unsorted CD8$^+$ population and the post-sorted samples (positive fraction, and negative fraction) are shown.

Supplementary Table 1. Recognition of autologous and allogeneic tumor cell lines by TIL derived from fresh tumor FrTu1913

		No target	TC1913	TC1913	FrTu#1913	FrTu#1913	TC624 CIITA	TC624 CIITA	TC624 CIITA	TC2119	TC2448	TC1865	TC1379	TC2301	OKT3[e]
			Aut.[a]	HLA-I block[b]	Aut.	HLA-I block	Allo. [c]	HLA-I block	HLA-II block[b]	Allo.	Allo.	Allo.	Allo.	Allo.	(0.1 µg/ml)
							A*0201[d]			A*0201	A*0201	A*0201	A*1101[d]		
TIL FrTu1913	CD8$^+$	31 (0.7)	77 (0.8)	0 (0.8)	398 (1.1)	83 (0.6)	14598 (2.2)	40 (0.8)	9626 (2.1)	2127 (0.7)	1845 (1.5)	318 (2.3)	1760 (2.0)	606 (1.1)	84824 (91.1)
	PD-1$^-$	0 (0.1)	0 (0.4)	0 (0.2)	27 (0.5)	13 (0.2)	11103 (2.9)	0 (0.5)	9986 (1.6)	78 (0.2)	105 (0.7)	1456 (3.0)	424 (1.9)	355 (1.2)	79037 (91.9)
	PD-1$^+$	11 (0.4)	26696 (42.2)	851 (16.9)	4108 (45.0)	363 (4.4)	0 (0.2)	0 (0.3)	0 (0.5)	1766 (1.6)	0 (0.8)	0 (0.3)	0 (0.3)	266 (2.1)	45319 (92.2)
	LAG-3$^-$	4 (0.4)	53 (n.d.)	0 (0.5)	225 (1.1)	45 (0.4)	17820 (3.7)	20 (0.9)	14872 (2.9)	532 (0.7)	91 (0.6)	922 (4.8)	808 (3.9)	571 (1.7)	78940 (91.6)
	LAG-3$^+$	0 (0.1)	55291 (49.0)	2345 (25.3)	6485 (45.7)	449 (4.3)	0 (0.1)	0 (0.1)	0 (0.1)	412 (0.8)	0 (0.1)	0 (0.3)	0 (0.2)	0 (0.1)	86689 (92.9)
	TIM-3$^-$	0 (0.1)	11 (0.6)	0 (0.1)	136 (0.6)	41 (0.2)	8092 (2.5)	0 (0.3)	6316 (1.6)	1614 (0.2)	467 (0.9)	1050 (4.1)	1167 (4.5)	160 (1.3)	>1666 (91.1)
	TIM-3$^+$	0 (0.1)	25472 (53.4)	1000 (17.9)	4761 (60.0)	310 (5.4)	0 (0.1)	0 (0.1)	0 (0.1)	3545 (2.0)	7 (1.6)	0 (0.1)	0 (0.1)	500 (1.5)	53519 (96.2)
Controls	F5 Td	24 (0.9)	47 (0.6)	43 (0.2)	148 (1.6)	96 (0.6)	22492 (59.0)	551 (14.1)	27567 (57.0)	33 (0.4)	13020 (58.1)	1056 (12.7)	15 (0.4)	21 (0.6)	81066 (98.4)
	CD4 T4	12 (0)	24 (0.4)	17 (0.3)	90 (0.3)	65 (0.3)	18831 (21.7)	20301 (20.0)	6305 (8.7)	0 (0)	0 (0)	3440 (8.0)	0 (0.1)	30 (0)	17949 (29.6)
	AK TIL1700	124 (1.1)	26 (1.4)	0 (0.6)	83 (1.1)	58 (0.5)	85 (2.1)	11 (0.5)	95 (2.0)	37 (1.3)	195 (4.2)	249 (2.2)	30 (0.9)	0 (0.6)	21976 (82.9)

Recognition of melanoma cell lines was assessed by evaluating IFN-γ release and upregulation of 4-1BB 24h after the coculture. Values represent the average pg/ml of IFN-γ released (duplicates). Percentage of CD3$^+$ CD8$^+$ 4-1BB$^+$ cells for each condition is shown in parenthesis. For CD4 T4, the values in parenthesis represent the CD3$^+$CD8$^-$4-1BB$^+$ cells. Control effectors: PBMC transduced with a T cell receptor recognizing Mart-1$_{26\text{-}35}$ epitope (F5 Td); PBMC transduced with a Tyrosinase$_{450\text{-}462}$ specific HLA-DR4-restricted T-cell receptor (CD4 T4). TC624 CIITA cell line is HLA-A*0201+ and HLA-DR4+ and expresses both MART1 and Tyrosinase. AK TIL1700 was used as a negative control.

[a] Autologous tumor cell line

[b] HLA-I block/HLA-II block denote the addition of HLA-I (W6/32) or HLA-II (IVA12) blocking antibodies during the coculture

[c] Allogeneic tumor cell line

[d] Cell line matched for the particular allele specified with tumor cell line TC1913

[e] Plate-bound anti-CD3 stimulation

Supplementary Table 2. Recognition of autologous and allogeneic tumor cell lines by TIL derived from tumor FrTu3289

		No target	**TC3289**	**TC3289**	**TC1379**	**TC526**	**TC526**	**CD4$^+$ CD25$^-$**	**OKT3[e]**
			Aut.[a]	**HLA-I block[b]**	**Allo.[c]**	**Allo.**	**HLA-I block**	**Aut. normal[d]**	**(0.1 µg/ml)**
TIL 3289	CD8$^+$	0 (0.4)	273 (4.5)	0 (0.4)	0 (0.6)	141 (1.6)	0 (0.2)	0 (1.3)	34289 (87.7)
	PD-1$^-$	5 (0.2)	6 (0.8)	0 (0.1)	752 (3.0)	117 (0.9)	0 (0.1)	0 (0.9)	45225 (86.9)
	PD-1$^+$	5 (0.3)	588 (16.2)	0 (0.3)	0 (4.5)	954 (5.8)	0 (0.1)	0 (2)	14491 (77.7)
	LAG-3$^-$	11 (0.3)	54 (2.8)	0 (0.2)	0 (0.6)	0 (0.6)	0 (0.2)	0 (1.1)	43907 (86.9)
	LAG-3$^+$	0 (0.6)	1040 (17.9)	0 (0.4)	0 (2.0)	229 (3.2)	0 (0.3)	0 (2.4)	27939 (83.4)
	TIM-3$^-$	0 (0.2)	0 (0.6)	0 (0.1)	26 (0.4)	0 (0.6)	0 (0.2)	0 (1.2)	49664 (85.5)
	TIM-3$^+$	0 (0.5)	1116 (17)	0 (0.4)	0 (1.1)	167 (2.1)	0 (0.2)	0 (2.3)	81743 (88.1)
Controls	F5 Td	0 (0.5)	8597 (18.4)	0 (0.2)	0 (0.4)	21684 (69.1)	739 (12.2)	165 (3.9)	73455 (86.3)
	AK TIL1700	246 (1.3)	30 (0.8)	12 (0.7)	33 (0.8)	117 (1.1)	3 (0.5)	231 (2.8)	19684 (88)

Recognition of melanoma cell lines was assessed by evaluating IFN-γ release and upregulation of 4-1BB 24 h after the coculture. Values represent the average pg/ml of IFN-γ released (duplicates). Percentage of CD3$^+$CD8$^+$4-1BB$^+$ cells for each condition is shown in parenthesis. Control effectors: PBMC transduced with a T cell receptor recognizing Mart-1$_{26\text{-}35}$ epitope (F5 Td); AK TIL1700 was used as a negative control. TC526 cell line is HLA-A*0201+ and expresses MART1.

[a] Autologous tumor cell line

[b] HLA-I block denotes the addition of HLA-I blocking (W6/32) antibody during the coculture

[c] Allogeneic tumor cell line

[d] Autologous CD4$^+$ CD25$^-$ cells were used as normal target cells

[e] Plate bound anti-CD3 stimulation

Supplementary Table 3. Recognition of autologous and allogeneic tumor cell lines by TIL derived from tumor FrTu3713

		No target	TC3713 Aut. [a]	TC3713 HLA-I block[b]	TC624 CIITA Allo. [c] A*0201 [d]	TC624 CIITA HLA-I block	TC624 CIITA HLA-II block[b]	TC2119 Allo. A*0201	TC2119 HLA-I block	TC1379 Allo. A*0201	TC3460 Allo. A*0201	OKT3[e] (0.1μ/ml)
TIIL FrTu3713	$CD8^+$	23 (0.1)	3242 (5.4)	78 (2.8)	731 (0.8)	38 (0.1)	504 (0.4)	174 (0.6)	17 (0.1)	66 (0.4)	34 (0.1)	43093 (52.0)
	$PD\text{-}1^-$	20 (0.1)	91 (0.6)	8 (0.1)	618 (0.6)	35 (0.1)	695 (0.3)	390 (0.4)	20 (0.1)	92 (0.6)	63 (0.2)	56023 (66.0)
	$PD\text{-}1^+$	0 (0.3)	7787 (51.0)	405 (24.1)	27 (0.8)	64 (0.3)	0 (0.3)	0 (0.8)	0 (0.2)	0 (0.5)	0 (0.7)	17037 (61.2)
	$TIM\text{-}3^-$	9 (0.2)	457 (1.4)	38 (0.4)	247 (1.3)	76 (0.5)	215 (0.6)	140 (0.6)	12 (0)	153 (0.7)	40 (0.3)	57436 (58.8)
	$TIM\text{-}3^+$	0 (0.2)	9650 (54.1)	342 (19.2)	38 (0.9)	0 (0.3)	0 (0.4)	11 (0.5)	0 (0.2)	0 (0.7)	0 (0.6)	13933 (53.8)
Controls	F5 Td	39 (0.6)	65 (0.4)	31 (0.2)	2445 (19.2)	232 (0.6)	3978 (20.6)	112 (0.7)	68 (0.2)	30 (0.4)	2455 (0.6)	61253 (90.9)
	CD4 T4	0 (0.1)	0 (0.5)	0 (0.2)	3091 (11.6)	3436 (4.6)	182 (2.0)	8 (1.1)	0 (0.3)	0 (0.6)	38 (1.8)	4150 (33.6)
	AK TIL1700	9 (1.3)	15 (7.5)	0 (1.0)	38 (3.7)	7 (1.1)	77 (3.2)	23 (3.5)	18 (1.6)	0 (1.7)	46 (2.0)	2198 (78.1)

Recognition of melanoma cell lines was assessed by evaluating IFN-γ release and upregulation of 4-1BB 24 h after the coculture. Values represent the average pg/ml of IFN-γ released (duplicates). Percentage of $CD3^+$ $CD8^+$ 4-$1BB^+$ cells for each condition is shown in parenthesis. For CD4 T4, the values in parenthesis represent the $CD3^+CD8^-$4-$1BB^+$ cells evaluated 24h after coculture. Control effectors: PBMC transduced with a T cell receptor recognizing Mart-$1_{26\text{-}35}$ epitope (F5 Td); PBMC transduced with a $Tyrosinase_{450\text{-}462}$ specific HLA-DR4-restricted T-cell receptor (CD4 T4). TC624 CIITA is HLA-A*0201+ and HLA-DR4+ and expresses both MART1 and Tyrosinase; AK TIL1700 was used as a negative control.

[a] Autologous tumor cell line

[b] HLA-I block/HLA-II block denote the addition of HLA-I (W6/32) or HLA-II (IVA12) blocking antibodies during the coculture

[c] Allogeneic tumor cell line

[d] Cell line matched for the particular allele specified with tumor cell line TC3713

[e] Plate bound anti-CD3 stimulation

Supplementary Table 4. Recognition of autologous tumor and allogeneic tumor cell lines by TIL derived from tumor FrTu3550

		% $CD3^+$ $CD8^+$	No target	TC3550	TC3550	TC624	TC624	TC3460	TC3460	OKT3[e]
		Post- REP[a]		Aut. [b]	HLA-I block[c]	Allo. [d]	HLA-I block	Allo.	HLA-I block	(0.1µg/ml)
TIL FrTu3550	$CD3^+$	70	10 (0.4)	55 (8.1)	11 (0.1)	321 (5.9)	12 (0.1)	11 (0.1)	12 (0.1)	136493 (94.9)
	PD-1^-	38	18 (0.4)	20 (1.7)	12 (0.1)	50 (2.9)	21 (0.2)	95 (0.8)	76 (0.3)	63949 (79.4)
	PD-1^+	45	10 (0.6)	1178 (27.3)	15 (1.3)	86 (4.7)	13 (0.3)	39 (0.7)	25 (0.2)	39044 (91.5)
	LAG-3^-	52	13 (0.1)	16 (0.3)	17 (0)	38 (0.5)	23 (0.1)	128 (0.2)	99 (0.1)	42645 (75.2)
	LAG-3^+	97	13 0.2)	1256 (19.5)	13 (0.3)	136 (6.2)	12 (0.1)	11 (0.1)	11 (0.1)	22468 (75.5)
	TIM-3^-	52	16 (0.2)	15 (0.3)	13 (0)	31 (0.4)	19 (0.1)	456 (0.4)	355 (0.1)	46018 (72.0)
	TIM-3^+	99	11 (0.2)	1502 (18.4)	12 (0.1)	101 (7.4)	15 (0.1)	13 (0.1)	12 (0)	39419 (87.4)
Controls	AK TIL1700	82	10 (3.6)	0 (1.0)	0 (2.9)	23 (1.1)	0 (1.1)	0 (0.6)	0 (0.2)	21539 (90.1)
	F5 Td	97	0 (0.2)	0 (0.2)	0 (0.1)	9016 (53.8)	97 (14.7)	0 (0.4)	0 (0.1)	77948 (83.2)

Recognition of melanoma cell lines was assessed by evaluating IFN-γ release and upregulation of 4-1BB 24h after the coculture. Values represent the average pg/ml of IFN-γ released (duplicates). Percentage of $CD3^+CD8^+4\text{-}1BB^+$ cells for each condition is shown in parenthesis. Control effectors: PBMC transduced with a T cell receptor recognizing Mart-$1_{26\text{-}35}$ epitope (F5 Td); AK TIL1700 was used as a negative control. TC624 cell line is HLA-A*0201+ and expresses MART1.

[a] Cells were originally sorted from the fresh tumor gating on $CD3^+$, and the percentage of $CD3^+$ $CD8^+$ after expansion (Post-REP) is shown.

[b] Autologous tumor cell line

[c] HLA-I block denotes the addition of HLA-I (W6/32) blocking antibody during the coculture

[d] Allogeneic tumor cell line

[e] Plate bound anti-CD3 stimulation

Supplementary Table 5. Recognition of autologous tumor and allogeneic tumor cell lines by TIL derived from FrTu2448

		No target	TC2448 Aut.[a]	TC2448 HLA-I block[b]	TC2301 Allo.[c]	TC526 Allo. A*0201[d]	TC526 HLA-I block	CD4$^+$ CD25$^-$ Aut. normal[e]	OKT3[f] (0.1 µg/ml)
TIL FrTu 2448	CD8$^+$	0 (1.4)	0 (1.7)	0 (0.5)	0 (1.1)	205 (2.5)	0 (0.5)	60 (1.4)	43195 (92.2)
	PD-1$^-$	0 (0.3)	13 (1.8)	0 (0.2)	0 (0.5)	1252 (14.9)	9 (0.1)	3 (0.7)	35718 (75.7)
	PD-1$^+$	0 (1.2)	194 (14.5)	0 (0.4)	0 (1.1)	1807 (15.2)	0 (0.5)	0 (1.9)	19927 (95.4)
	LAG-3$^-$	0 (1.2)	0 (1.6)	79 (0.5)	22 (0.6)	15 (4.6)	0 (0.4)	34 (1.1)	28962 (68.8)
	LAG-3$^+$	11 (0.2)	0 (3.3)	0 (0.1)	0 (0.2)	82 (10.4)	0 (0.2)	0 (0.7)	65167 (97.1)
	TIM-3$^-$	38 (0.3)	0 (0.9)	0 (0.3)	83 (0.8)	69 (1.5)	0 (0.3)	245 (0.9)	76231 (94.7)
	TIM-3$^+$	0 (0.4)	543 (9.9)	0 (0.4)	0 (0.4)	2862 (9.7)	7 (0.4)	54 (2.0)	50990 (95.7)
	F5 Td	56 (0.6)	4101 (40.1)	175 (3.0)	70 (0.6)	18497 (73.3)	1373 (13.8)	242 90.8)	62741 (91.6)
	AK TIL1700	344 (0.8)	386 (4.2)	75 (1.0)	120 (1.1)	317 (1.9)	120 (0.8)	266 (1.2)	27753 (86.8)

Recognition of melanoma cell lines was assessed by evaluating IFN-γ release and upregulation of 4-1BB 24 h after the coculture. Values represent the average pg/ml of IFN-γ released (duplicates). Percentage of CD3$^+$CD8$^+$4-1BB$^+$ cells for each condition is shown in parenthesis. Control effectors: PBMC transduced with a T cell receptor recognizing Mart-1$_{26-35}$ epitope (F5 Td); AK TIL1700 was used as a negative control.

[a] Autologous tumor cell line.

[b] MHC I block denotes the addition of HLA-I (W6/32) blocking antibody during the coculture

[c] Allogeneic tumor cell line

[d] Cell line matched for the particular allele specified with tumor cell line TC2448

[e] Autologous CD4$^+$ CD25$^-$ cells were used as normal target cells

[f] Plate bound anti-CD3 stimulation

Supplementary Table 6. Recognition of autologous tumor and allogeneic tumor cell lines by TIL derived from FrTu3289

	No target		TC3289		TC3289		TC624 CIITA		TC1913		OKT3[d]		TC624 CIITA		TC624 CIITA	
			Aut.[a]				Allo. [c]		Allo.							
					HLA-I block[b]						0.1 µg/ml		HLA-I block		HLA-II block[b]	
CD8$^+$	18	(0.3)	107	(3.9)	24	(0.4)	81	(3.6)	27	(1.1)	32587	(91.9)				
PD-1$^-$	29	(1.0)	75	(4.8)	21	(1.5)	103	(4.4)	25	(2.7)	65554	(89.8)				
PD-1$^+$	13	(2.5)	2127	(31.7)	9	(2.2)	72	(6.5)	8	(4.1)	16910	(90.1)				
TIM-3$^-$	47	(0.2)	35	(1.3)	18	(0.3)	71	(2.8)	20	(0.7)	55642	(87.2)				
TIM-3$^+$	16	(1.2)	2361	(33.7)	30	(1.0)	123	(5.9)	19	(2.6)	18020	(95.1)				
LAG-3$^-$	23	(0.3)	20	(1.3)	17	(0.4)	126	(2.9)	32	(1.1)	55437	(87.5)				
LAG-3$^+$	6	(1.9)	2357	(34.2)	6	(2.1)	732	(13.8)	8	(3.7)	18206	(91.8)				
PD-1$^-$TIM-3$^-$	25	(0.6)	19	(2.4)	22	(0.8)	146	(4.3)	55	(2.0)	46080	(89.0)				
PD-1$^-$ TM-3$^+$	32	(1.2)	3411	(22.3)	33	(1.3)	732	(7.3)	63	(4.0)	50553	(88.8)				
PD-1$^+$TIM-3$^+$	24	(3.3)	2770	(45.2)	20	(3.9)	1175	(18.8)	16	(6.4)	34950	(82.9)				
PD-1$^+$TIM-3$^-$	30	(1.5)	215	(16.1)	16	(1.8)	488	(12.8)	18	(4.1)	17694	(75.0)				
PD-1$^-$LAG-3$^-$	38	(0.7)	29	(2.1)	19	(0.7)	106	(5.3)	33	(1.6)	21707	(90.3)				
PD-1$^-$LAG-3$^+$	24	(1.3)	438	(20.6)	22	(1.2)	62	(5.0)	19	(2.2)	20877	(92.6)				
PD-1$^+$LAG-3$^+$	16	(3.9)	2409	(42.4)	12	(5.8)	277	(14.6)	20	(8.5)	26653	(94.6)				
PD-1$^+$LAG-3$^-$	11	(1.8)	286	(13.3)	12	(3.1)	846	(11.2)	17	(3.6)	55811	(89.8)				
TIL1913	11	(0.3)	41	(2.7)	13	(0.4)	8	(0.9)	10017	(59.4)						
CD4 T4	0	(0.1)					>16666	(34.4)	0	0.3	13815	(45.1)	>16666	(31.0)	195	(1.7)
F5 Td	6	(4.8)					4088	(21.4)	0	5.2	13283	(93.8)	36	(8.4)	3236	(18.2)

CD8$^+$ TIL populations were sorted from FrTu3289 according to PD1, LAG-3 and TIM-3 expression and expanded in vitro for 15 days. Recognition of melanoma cell lines was assessed by evaluating IFN-γ release and upregulation of 4-1BB 24 h after the coculture. Values represent the average pg/ml of IFN-γ released (duplicates). Percentage of CD3$^+$CD8$^+$4-1BB$^+$ cells for each condition is shown in parenthesis (duplicates). For CD4 T4, the values in parenthesis represent the CD3$^+$CD8$^-$4-1BB$^+$ cells. Control effectors: TIL grown derived from FrTu1913 (TIL1913); PBMC transduced with a T-cell receptor recognizing MART1$_{26\text{-}35}$ epitope (F5 Td); PBMC transduced with a Tyrosinase$_{450\text{-}462}$ specific HLA-DR4-restricted T-cell receptor (CD4 T4). TC624 CIITA is HLA-A*0201+ and HLA-DR4+ and expresses both MART1 and Tyrosinase.

[a] Autologous tumor cell line

[b] HLA-I block/HLA-II block denote the addition of HLA-I (W6/32) or HLA-II (IVA12) blocking antibodies during the coculture

[c] Allogeneic tumor cell line

[d] Plate-bound anti-CD3 stimulation

Supplementary Table 7. Patient characteristics

variable/trait	Total (%)
Total no. patients	24 (100)
Sex	
Male	19 (79)
Female	5 (21)
Age	
11-20	2 (8)
21-30	2 (8)
31-40	5 (21)
41-50	4 (17)
51-60	10 (42)
61-70	1 (4)
Prior Treatment	
Surgery	22 (92)
Chemotherapy	3 (13)
Radiotherapy	2 (8)
Immunotherapy	19 (79)
Any 2 or more	17 (71)
Any 3 or more	4 (17)
No treatment	2 (8)

Supplementary Table 8. Immunotherapeutic regimens prior to tumor resection

Tumor sample	Prior Immunotherapy	Months prior to resection	Site of tumor resection
FrTu1913	Cisplatin, vinblastine, dacarbazine, IL-2[a], IFN[b]	8	Subcutaneous
	MART-1: 26-35(27L)/gp100: 209-2017 (210M)+IL-2[c]	2	
FrTu3289	Cisplatine, vinblastine, dacarbazine, IL-2	54	Spleen
FrTu3612	IL-2	4	Mesentheric/small bowel
FrTu3713	IL-2	6	Lung
	anti-CTLA-4[d]	2	
FrTu3550	-		Axillary LN
FrTu2448	IL-7[e]	2	Inguinal LN

[a] Interleukin 2

[b] Interferon

[c] Immunization by subcutaneous injection of the peptides specified emulsified in incomplete Freund’s adjuvant and IL-2

[d] Anti-cytotoxic T-lymphocyte antigen 4 blocking antibody

[e] Interleukin 7

Supplementary Table 9. Fold-expansion of TIL sorted and expanded from fresh tumor digests.

	Fold expansion									
	CD8$^+$	**PD-1$^-$**	**PD-1$^+$**	**LAG-3$^-$**	**LAG-3$^+$**	**TIM-3$^-$**	**TIM-3$^+$**	**PD-1$^-$/4-1BB$^-$**	**PD-1$^+$/4-1BB$^-$**	**PD-1$^+$/4-1BB$^+$**
FrTu1913	3200	4400	4400	4400	73807	4583	7000			
FrTu2448	5078	2916	44332	2156	127871	2105	68966			
FrTu3289	3275	3650	4700	4400	6643	4200	5200			
FrTu3550[a]	643	311.5	793.6	277	828	285	1280			
FrTu3713	2867	3067	3333			5450	15000	714	1811	2080
FrTu3612	1720	495	1651							

Cells were FACS sorted to high purity from the fresh tumor digests specified and then expanded with anti-CD3 stimulation, IL-2, and irradiated feeders. Fold-expansion of the cells after 15 days in culture is shown.

[a] Cells from FrTu3550, were sorted based on CD3 expression, and fold-expansion corresponds to CD3$^+$ T cells