Supplementary Figure 1. Activation of RhoC downstream of ErbB-2 is mediated by Plexin-B1. HEK293 cells were transfected with VSV-Plexin-B1 as well as HA-RhoC and FLAG-PDZ-RhoGEF. Where indicated, cells were additionally transfected with wildtype ErbB-2 (ErbB-2 WT), or a Plexin-B1 deletion construct which lacks the intracellular domain (PlxB1ΔC). After incubation without or with 150 nM Sema4D for 20 min, active RhoC was precipitated (pulldown) and precipitates were immunoblotted (IB) using an anti-HA-antibody.

Supplementary Figure 2. (**A**) Knockdown of ErbB-2 does not affect active R-Ras levels. BT-474 cells were transfected with control or ErbB-2 siRNA. 48h later, cells were lysed, active R-Ras was immunoprecipitated (pulldown) and precipitates were immunoblotted using an anti-R-Ras antibody. (**B-C**) Stable knockdown of Plexin-B1 impairs migration and invasion of BT-474 cells. Using a lentiviral system, BT-474 cells were stably transfected with control shRNA or shRNAs directed against Plexin-B1. (**B**) 24 h after seeding onto non-coated filters or (**C**) 48h after seeding onto Matrigel-coated filters, cells on the upper side of the filters were removed and cells on the bottom side of the filter were counted as described in Methods. (**D**) SiRNA-mediated knockdown of RhoA or RhoC reduces invasiveness of BT-474 cells. BT-474 cells transfected with control, RhoA or RhoC siRNA were seeded onto Matrigel-coated filters. 48h later, non-invading cells were removed and invading cells were counted. (**E-H**) The mouse monoclonal anti-Plexin-B1 antibody (clone #93) interferes with the interaction between ErbB-2 and Plexin-B1, but does not inhibit binding of Sema4D to Plexin-B1. Uncoupling of ErbB-2 and Plexin-B1 by the mouse monoclonal anti-Plexin-B1 antibody (clone #93)

or the soluble extracellular domain of Plexin-B1 (PlxB1ext) reduces tyrosine phosphorylation of Plexin-B1. (**E**) BT-474 cells were incubated with the indicated concentrations of a mouse monoclonal anti-Plexin-B1 antibody (anti-PlxB1; clone #93) for 60 min. Thereafter, cells were lysed, ErbB-2 was immunoprecipitated using an anti-ErbB-2 antibody (IP) and Plexin-B1 was immunoprecipitated using an anti-Plexin-B1 antibody (R&D Systems; IP). Precipitates were immunoblotted (IB) with antibodies directed against Plexin-B1 (R&D Systems), phospho-tyrosine (pTyr), or ErbB-2. (**F**) HEK293 cells were transfected with 3DA.Luc and plasmids encoding Plexin-B1 and PDZ-RhoGEF. 3DA.Luc represents a reporter plasmid expressing firefly luciferase under the control of a mutant serum response element (SRE) which is activated downstream of active RhoA. After incubation with the indicated concentrations of the mouse monoclonal anti-Plexin-B1 antibody (clone #93) for 60 min, cells were treated with 150 nM Sema4D for 4h and firefly luciferase activity corresponding to RhoA activity was determined as described in materials and methods. (**G**) MCF-7 cells expressing endogenous Plexin-B1 were incubated with the indicated concentrations of the mouse monoclonal anti-Plexin-B1 antibody (clone #93) for 1 hour. After washing with PBS, cells were treated with myc-Sema4D for 30 min. After removal of unbound myc-Sema4D by washing, cells were incubated with an HRP-conjugated anti-myc antibody for 30 min, washed, and HRP-activity was determined as described in materials and methods. (**H**) BT-474 cells were incubated with the indicated concentrations of the soluble extracellular domain of Plexin-B1 (PlxB1ext) for 45 min. Thereafter, cells were lysed, Plexin-B1 was immunoprecipitated using an anti-Plexin-B1 antibody (R&D

Systems; IP) and precipitates were immunoblotted (IB) with antibodies directed against Plexin-B1 (R&D Systems), phospho-tyrosine (pTyr), or ErbB-2.

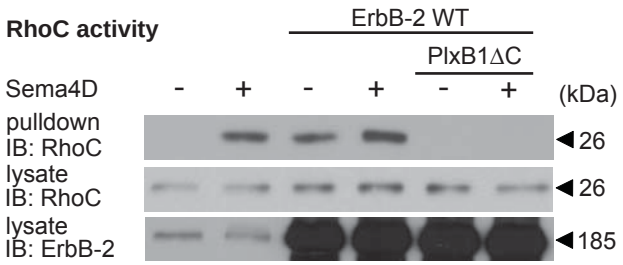
Supplementary Figure 3. Plexin-B1 promotes invasion of ErbB-2-overexpressing human ovarian cancer cells. (**A**) SK-OV-3 cells were incubated with the indicated concentrations of a mouse monoclonal anti-Plexin-B1 antibody (anti-PlxB1; clone #93) for 60 min. After cell lysis ErbB-2 was immunoprecipitated using an anti-ErbB-2 antibody (IP) and Plexin-B1 was immunoprecipitated using an anti-Plexin-B1 antibody (R&D Systems; IP). Precipitates were immunoblotted (IB) with antibodies directed against phospho-tyrosine (pTyr), ErbB-2 or Plexin-B1. (**B**) SK-OV-3 cells were incubated without or with a mouse monoclonal anti-Plexin-B1 antibody (anti-PlxB1; clone #93, 1.8 ng/µl) for 60 min. Thereafter, cells were lysed and the amounts of active RhoA and RhoC were determined as described (pulldown). (**C**) SK-OV-3 cells were seeded onto Matrigel-coated filters in the absence or presence of a mouse monoclonal anti-Plexin-B1 antibody (anti-PlxB1; clone #93, 1.8 ng/µl). 16h later, non-invading cells were removed, and invading cells were counted. Data are presented as mean ± S.D. with statistical significances determined by t-test; **, $p \leq 0.01$.

Supplementary Figure 4. Analysis of MMTVneu primary tumors. (**A**) RNA from primary tumor and lung metastasis was isolated and reverse transcribed (RT, reverse transcriptase). PCR analysis was carried out using primers specific for Plexin-B1. Primers for beta-actin were used as control. (**B,C**) Apoptosis, (**D,E**) vascularization, (**F,G**) macrophage infiltration, (**H,I**) phospho-ErbB-2 score, (**J,K**) grading and (**L,M**) local

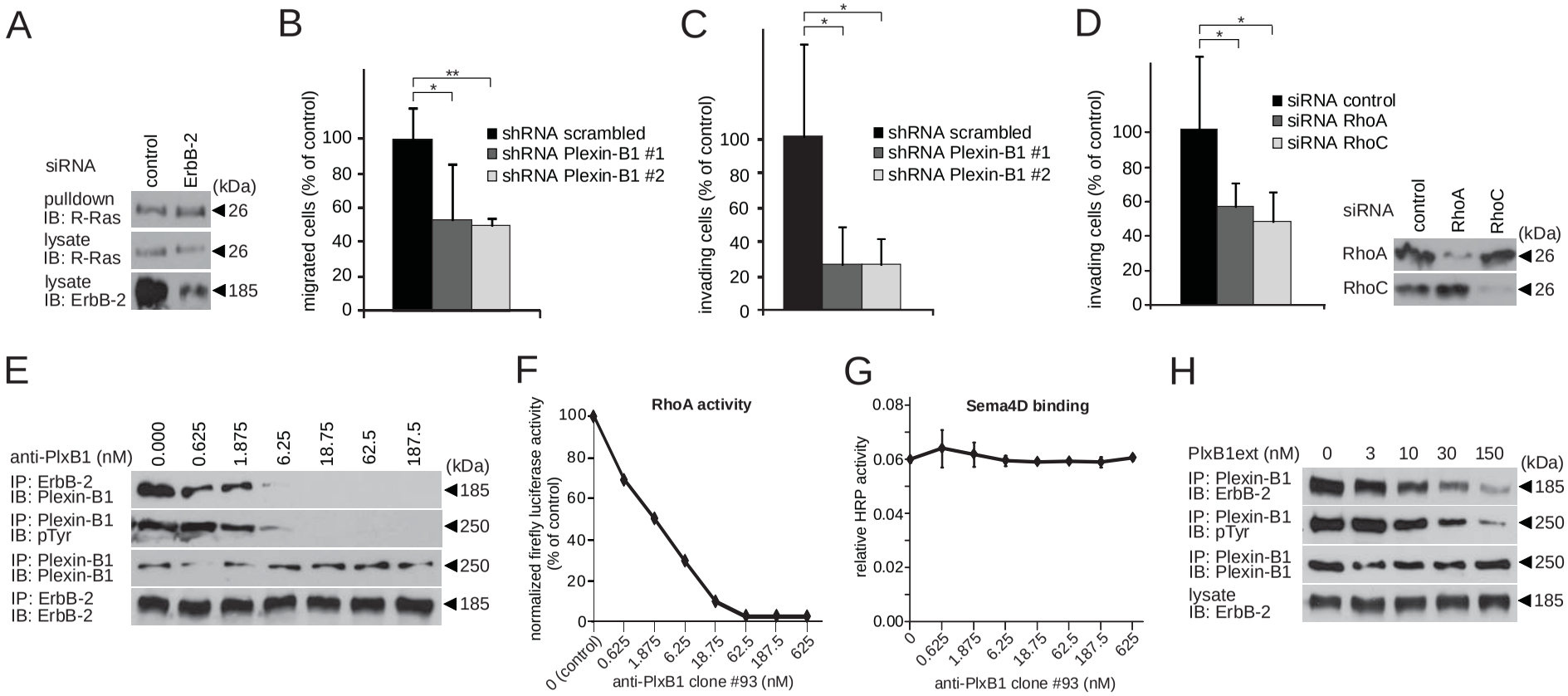
invasiveness **(Plexin-B1 WT, n = 7; Plexin-B1 KO, n = 13)** of MMTVneu primary tumors. Representative pictures are shown in (**B,D,F,H,J,L**)**,** quantifications of the results are provided in (**C,E,G,I,K,M**)**.** Arrows point to (**B**) apoptotic cells positive for cleaved-caspase-3 (blue), (**D**) CD-31-positive blood vessels (red), (**F**) Mac-3-positive macrophages (red), (**J**) mitotic figures. The invasion front is marked by white dashed lines in (**L**). Scale bars represent 200 µm in (**D**), 40 µm in (**H**), 20 µm in (**B,F,J,L**)**.**

Supplementary Figure 5. Kaplan-Meier graphs representing the disease-free survival of patients with (**A**) ErbB-2-negative breast cancer (Plexin-B1 high: n=62, Plexin-B1 low: n=19), (**B**) ErbB-2-positive, ER-positive breast cancer (Plexin-B1 high: n=15, Plexin-B1 low: n=15), (**C**) ErbB-2-positive, ER-negative breast cancer (Plexin-B1 high: n=14, Plexin-B1 low: n=14). Black, high Plexin-B1 expression (Plexin-B1 high); grey, low Plexin-B1 expression (Plexin-B1 low). Statistical significances were tested by log-rank-test.

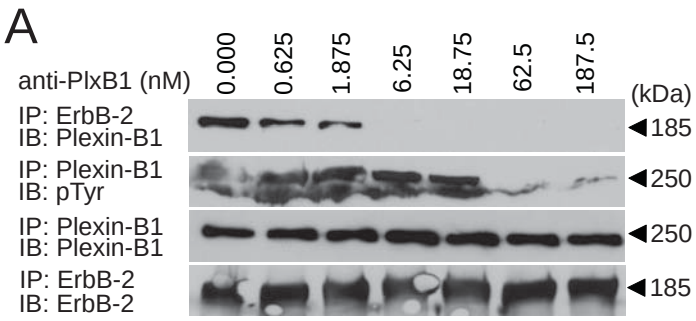
Supplementary Table 1. Characteristics of breast cancer patients of whom (**A**) frozen breast cancer tissue was obtained for RT-PCR, (**B**) paraffin-embedded breast cancer tissue was obtained for immunohistochemistry, (**C**) frozen breast cancer tissue was obtained for immunoprecipitation and Western Blot.

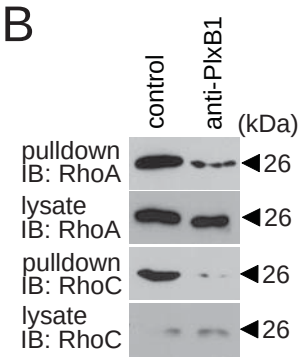


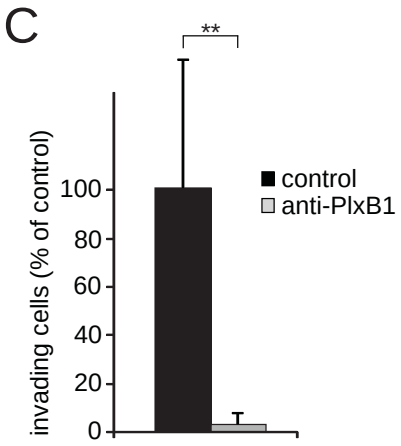
Supplementary Fig. 1, Worzfeld et al.



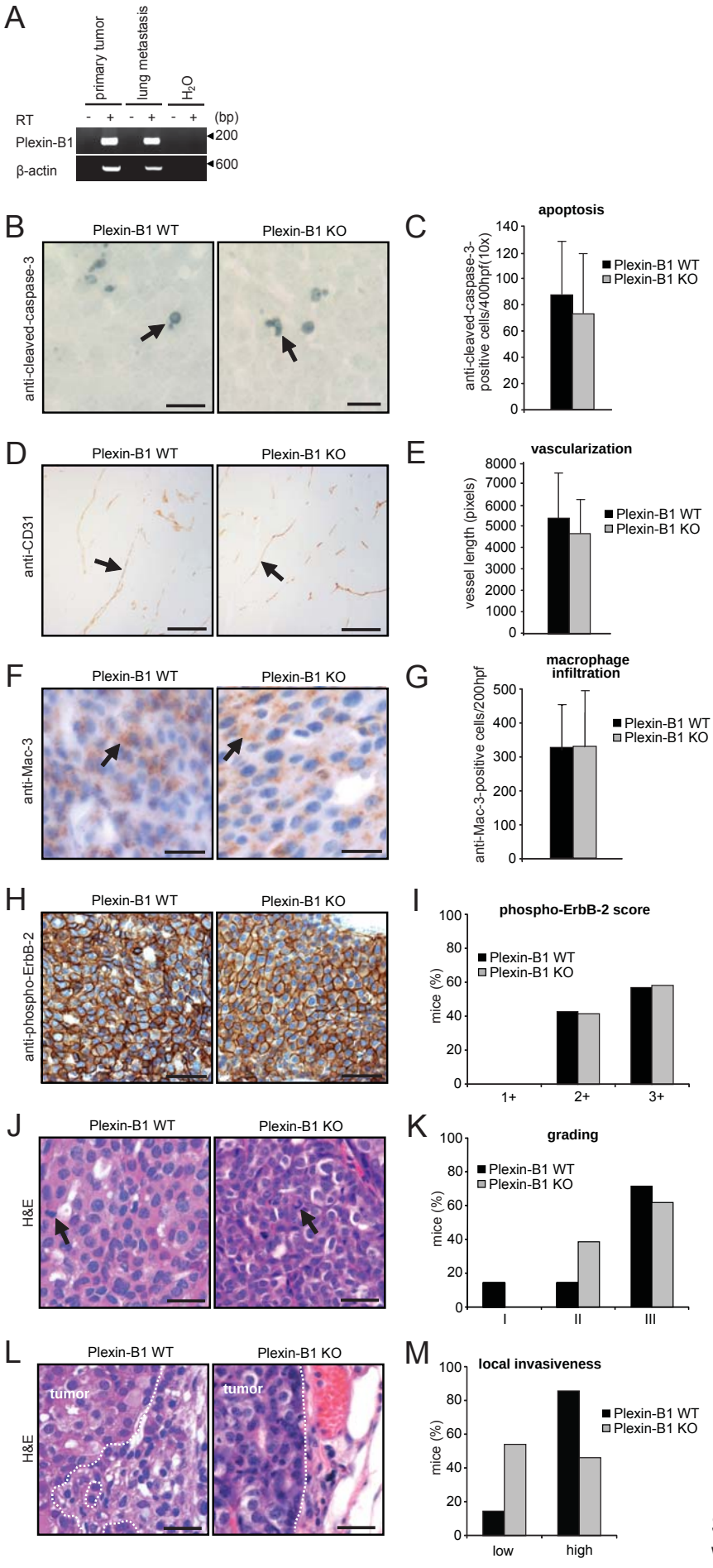
Supplementary Figure 2, Worzfeld et al.



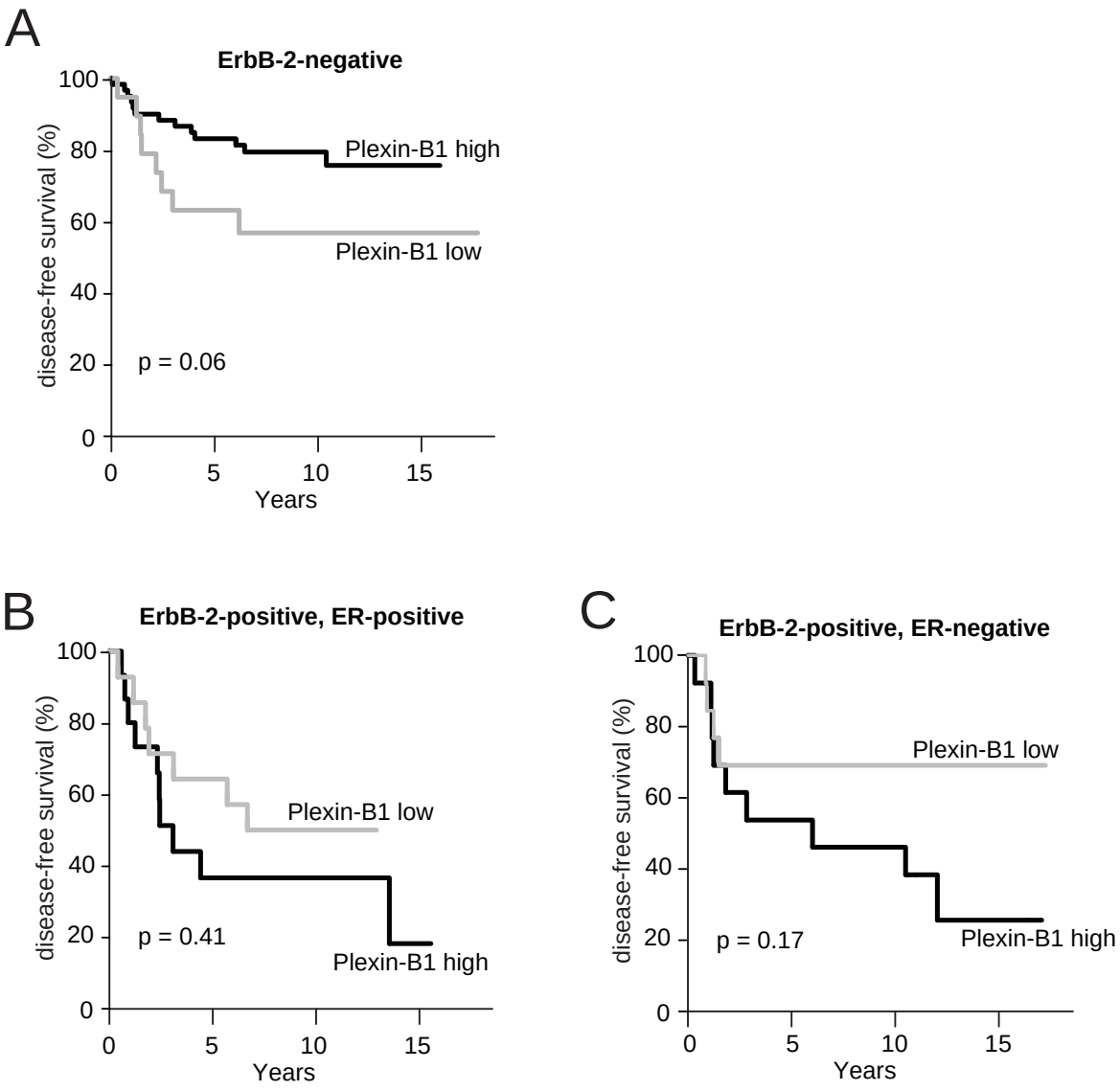




Supplementary Figure 3, Worzfeld et al.



Supplementary Figure 4,
Worzfeld et al.



Supplementary Figure 5, Worzfeld et al.

A

patient ID	DOB	histopathology	T	N	grade	ErbB-2 score
2005	05/26/1954	invasive ductal carcinoma	1c	0 (0/19)	3	0
2030	03/13/1928	invasive ductal carcinoma	1c	0 (0/23)	1	0
2051	07/01/1930	invasive lobular carcinoma	3 (m)	2	3	0
1900	08/14/1941	invasive ductal carcinoma	2	0 (0/22)	3	3+
1935	06/14/1953	invasive ductal carcinoma	2	0 (0/21)	3	3+
2004	11/27/1940	invasive ductal carcinoma	2	1 bi (1/21)	3	3+

B

patient ID	histopathology	ErbB-2 score
3541	invasive ductal carcinoma	0
16897	invasive ductal carcinoma	3+

C

patient ID	histopathology	grade	ErbB-2 score
372	invasive lobular carcinoma	G2	0
664	invasive ductal carcinoma	G2	0
1635	invasive medullary carcinoma	G3	0
4738	invasive ductal carcinoma	G2	0
5499	invasive ductal carcinoma	G2	1+
654	invasive ductal carcinoma	G2	1+
3531	invasive ductal carcinoma	G2	2+
4353	invasive ductal carcinoma	G2	3+
1610	invasive medullary carcinoma	G3	3+
102	invasive ductal carcinoma	G2	0
934	invasive lobular carcinoma	G2	0
851	invasive ductal carcinoma	G2	0
5809	invasive ductal carcinoma	G2	0
3405	invasive mucinous carcinoma	G2	1+
1048	invasive ductal carcinoma	G3	1+
3156	invasive ductal carcinoma	G3	2+
5939	invasive ductal carcinoma	G2	3+
4017	invasive ductal carcinoma	G2	3+

Supplementary Table 1, Worzfeld et al.