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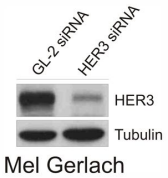

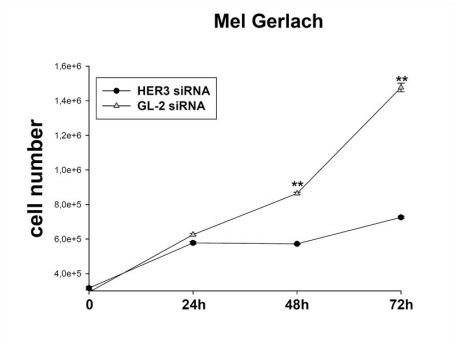

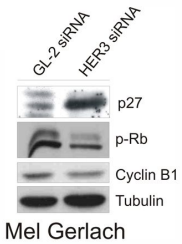

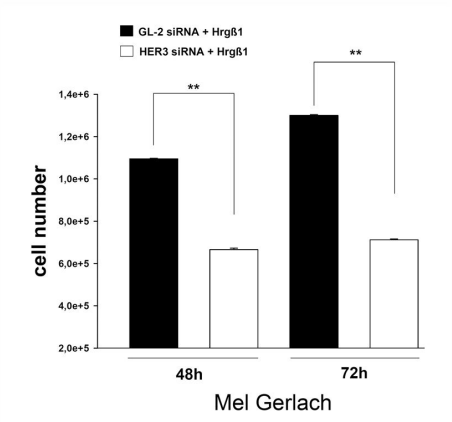

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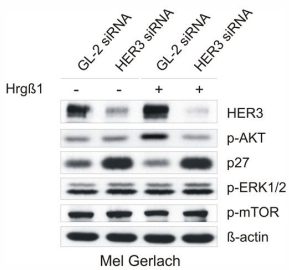

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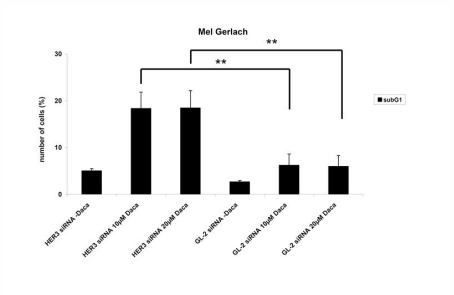

Supplementary Figure 1

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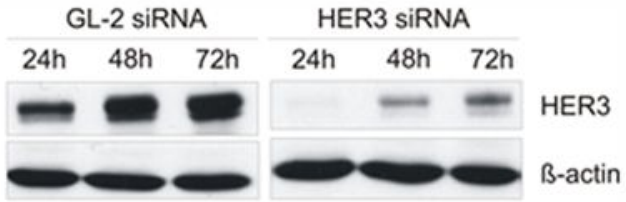

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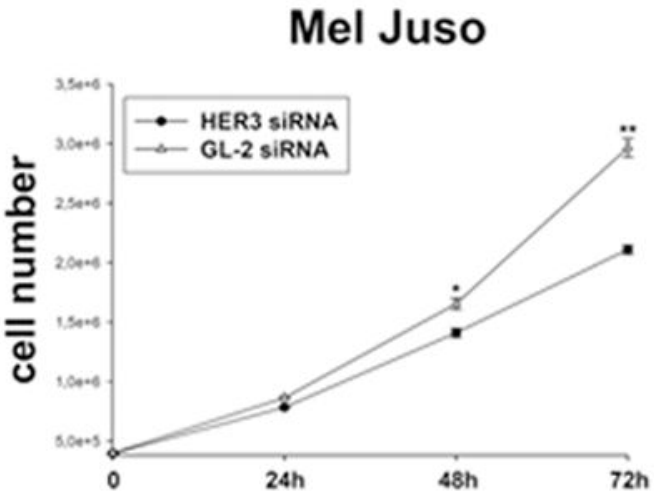

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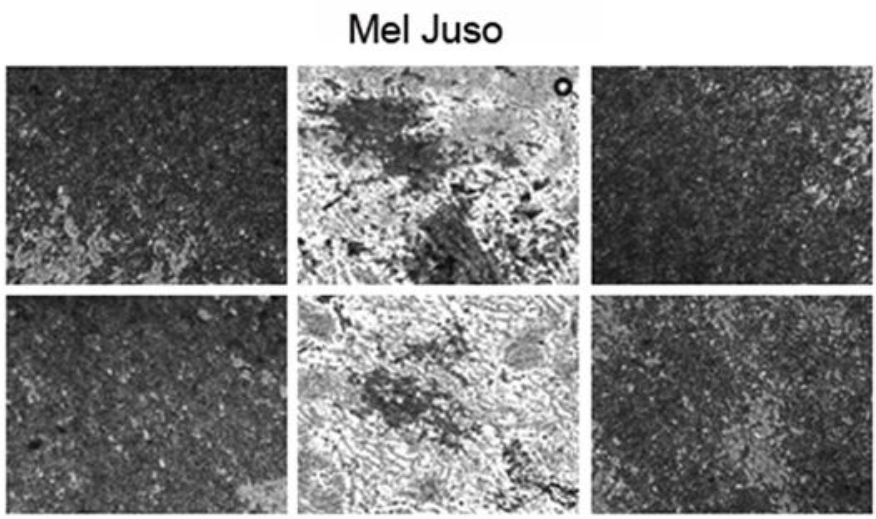
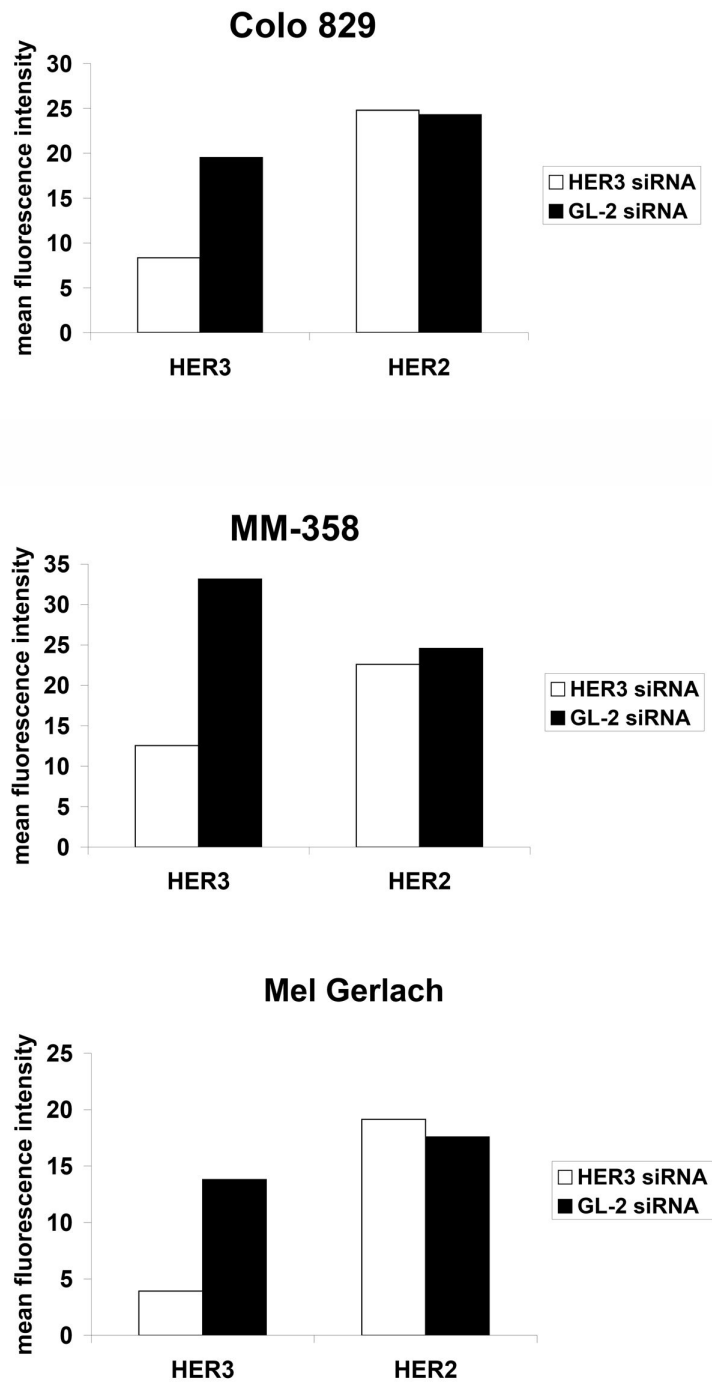
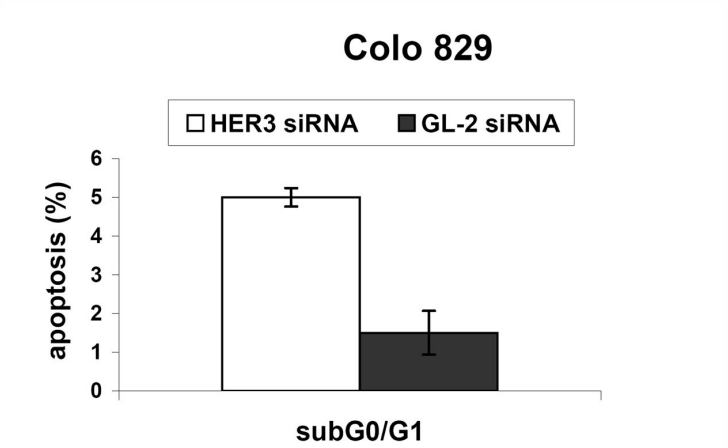
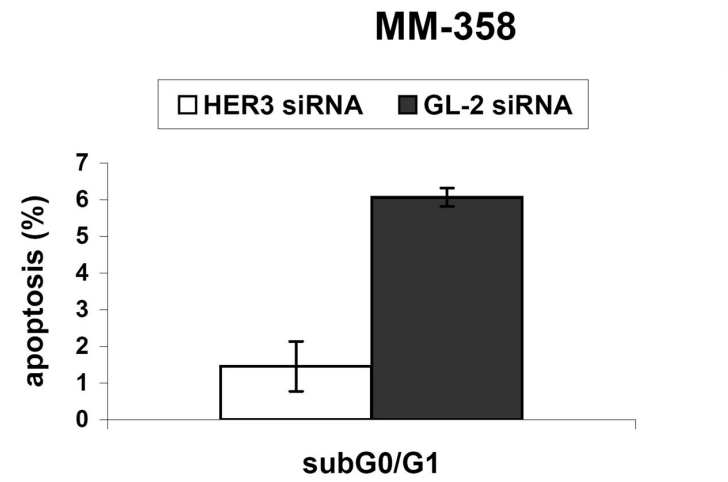


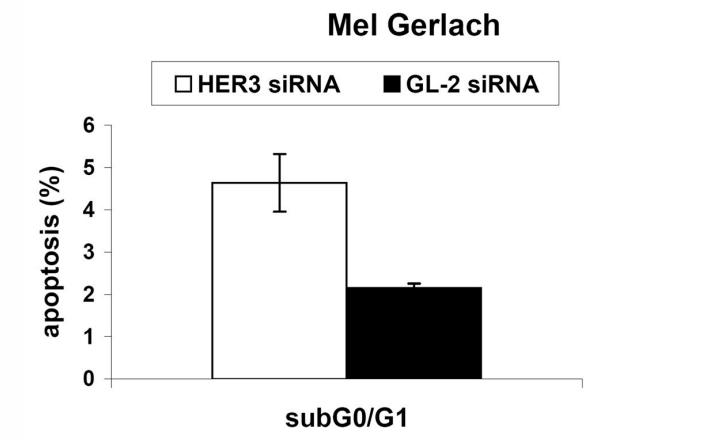
Reschke et al. Supplementary Figure 2



Reschke et al. Supplementary Figure 3







Reschke et al. Supplementary Figure 4

Supplementary Data

HER3 is a Determinant for Poor Prognosis in Melanoma

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Supplementary Fig. 1 *HER3* knock-down in Mel Gerlach melanoma cells.

A *HER3* knockdown inhibits the proliferation and heregulin-induced proliferation of Mel Gerlach melanoma cells. *HER3* knock-down and *GL-2* control cells were either grown in the presence of 10% FCS or serum-starved in medium containing 1% FCS and stimulated with 100ng/ml heregulin ß1. The cells were counted at the indicated time points and data are shown as mean ± SDM. Western Blots for HER3, p27, p-Rb and CyclinB1 are shown. Tubulin served as a loading control.

B. *HER3* knock-down impairs heregulin-induced AKT activity in Mel Gerlach melanoma cells. Western blots for HER3, p-AKT, p27, p-ERK1/2 and p-mTOR are shown. ß-actin served as loading control. **C** *HER3* knock-down sensitizes Mel Gerlach melanoma cells to dacarbazine-induced apoptosis. Mel Gerlach *HER3* knock-down and *GL-2* control cells were either treated with 10 or 20µM dacarbazine or left untreated for 48 hours and apoptosis was analyzed by flow cytometry.

Supplementary Fig. 2 *HER3* knock-down in Mel Juso melanoma cells A *HER3* knock-down in Mel Juso melanoma cells. Cells were lysed at the indicated time points and subjected to Western blot analysis for HER3. ß-actin served as a loading control. **B** *HER3* knock-down inhibits the proliferation of Mel Juso melanoma cells. **C** An anti-HER3 monoclonal antibody (cl. 2D1D12) inhibits heregulin-induced invasion of Mel Juso melanoma cells. Mel Juso cells were either incubated with an anti-HER3 monoclonal antibody (cl. 2D1D12; 10µg/ml), an anti-HER2 monoclonal antibody (4D5; 10µg/ml) or left untreated.

Supplementary Fig. 3 *HER3* knock-down does not alter HER2 surface expression in melanoma cell lines A-C HER3 and HER2 surface expression was measured by indirect flow cytometry. Colo 829, Mel Gerlach and MM-358 *HER3*

knock-down cells were incubated with specific primary antibodies for HER2 and HER3 for 1 hour and afterwards with a PE-labeled secondary antibody followed by flow cytometry. The fluorescence intensities for HER3 and HER2 are shown.

Supplementary Fig. 4 *HER3* knock-down does not induce apoptosis in melanoma cells 200.000 cells were seeded in 6 well plates and transfected with *HER3* or *GL-2* siRNAs using oligofectamine (Invitrogen). Cells were trypsinized after 48 hours and analyzed by Propidium Iodide staining as described in Material and Methods. Apoptotic cells were identified as the subG0/G1 population and quantified using the Cell Quest Pro software (Beckton Dickinson Biosciences).

Indirect flow cytometry

The antibodies used for HER3 and HER2 were described elsewhere (18, 19). 500.000 cells were seeded in 10 cm dishes (Falcon) and transfected with *HER3* or *GL-2* siRNAs using oligofectamine (Invitrogen). Cells were collected after 24 hours using 10mM EDTA and dissolved in 1ml 3% FCS in PBS. The cell number was adjusted to 250.000 cells per reaction and cells were incubated for 30 minutes with 10µg/ml of each primary antibody at 4°C. The cells were washed 3 times in 3% FCS/PBS and incubated with a PE-labeled secondary (1:1000) antibody at 4°C for 30 minutes. After three more washes in 3% FCS/PBS the fluorescence intensity was measured in a flow cytometer (Beckton Dickinson Biosciences) and analyzed using the Cell Quest Pro software.