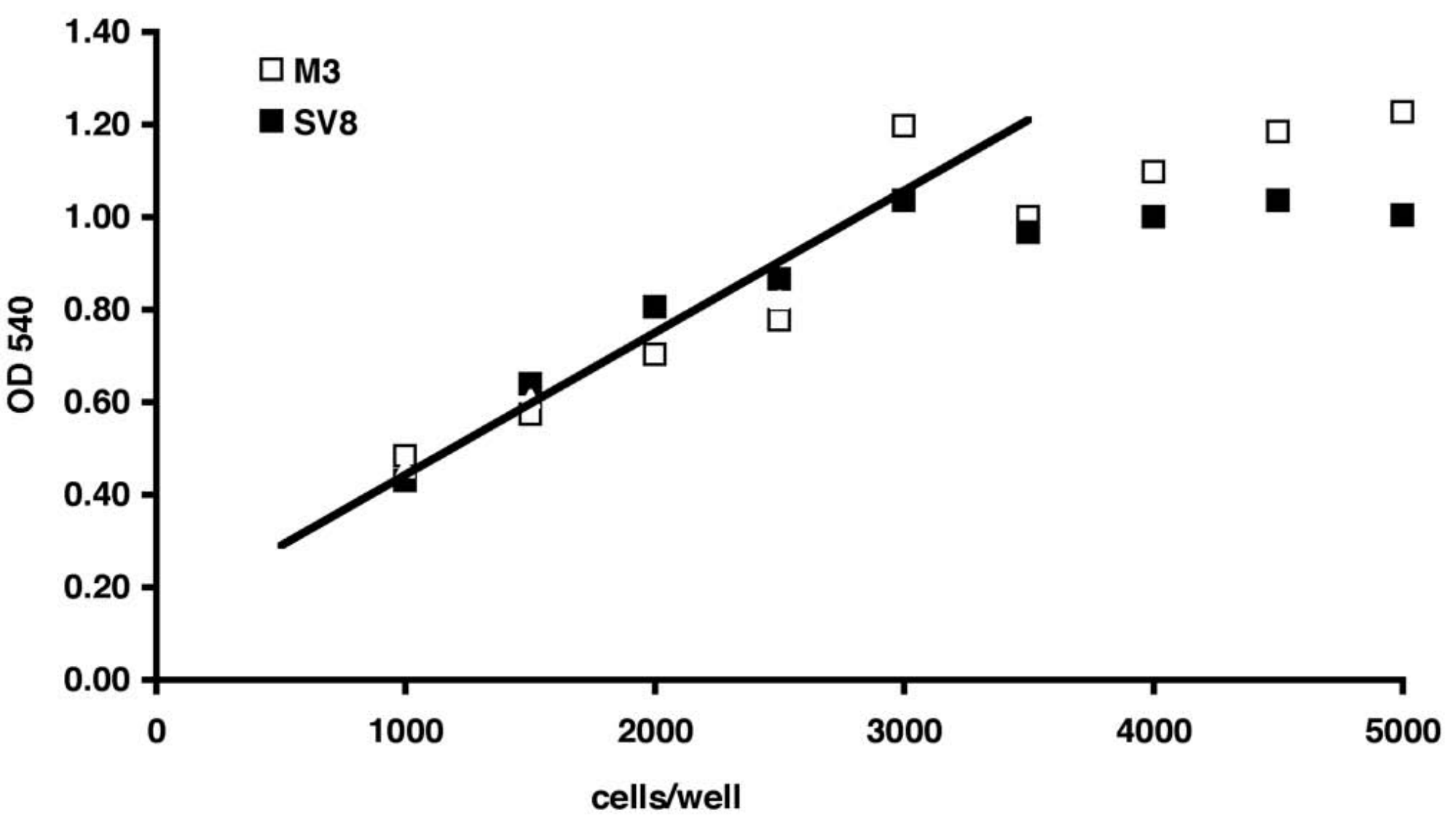
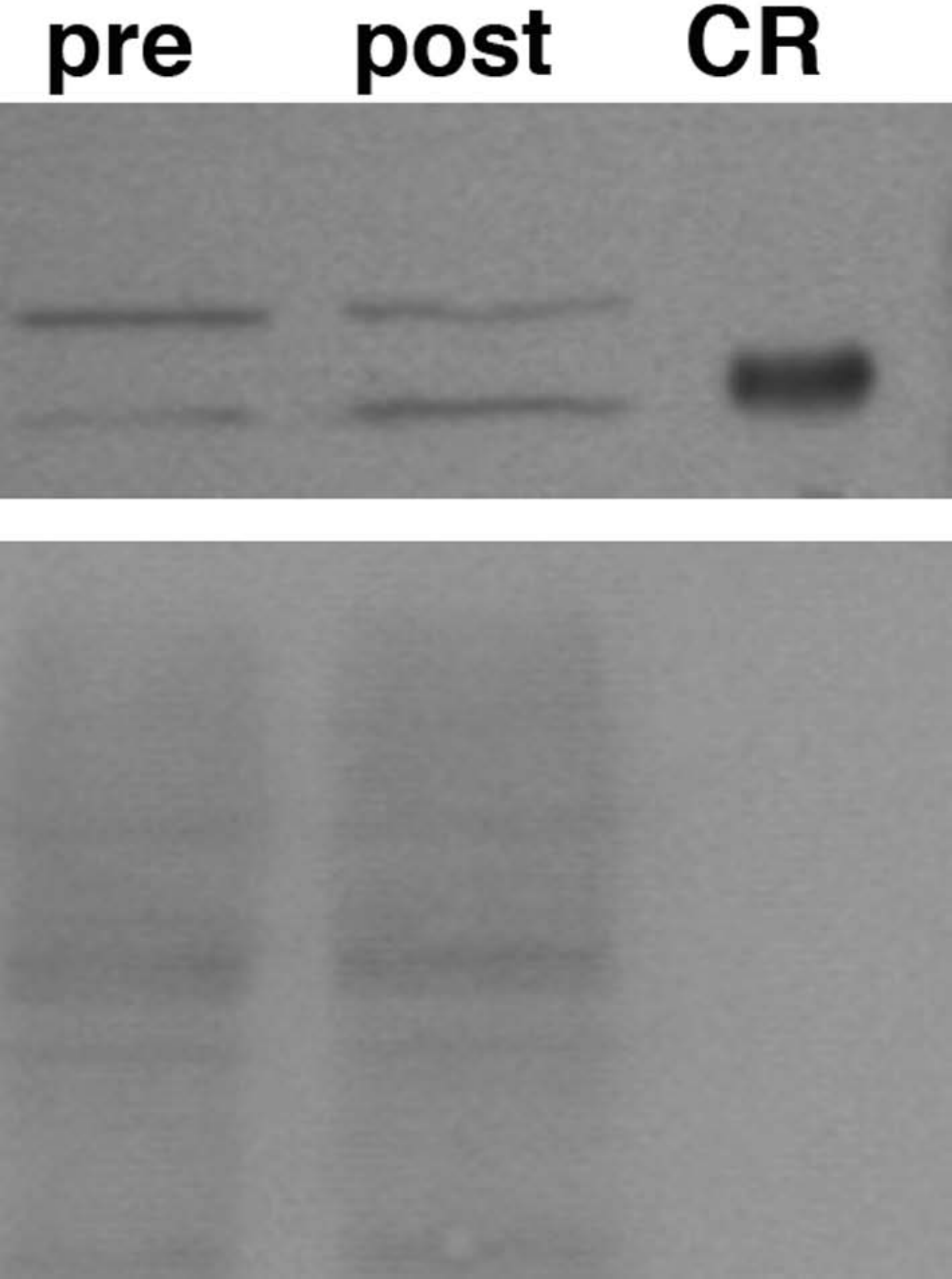
Legends to Supplemental Figures

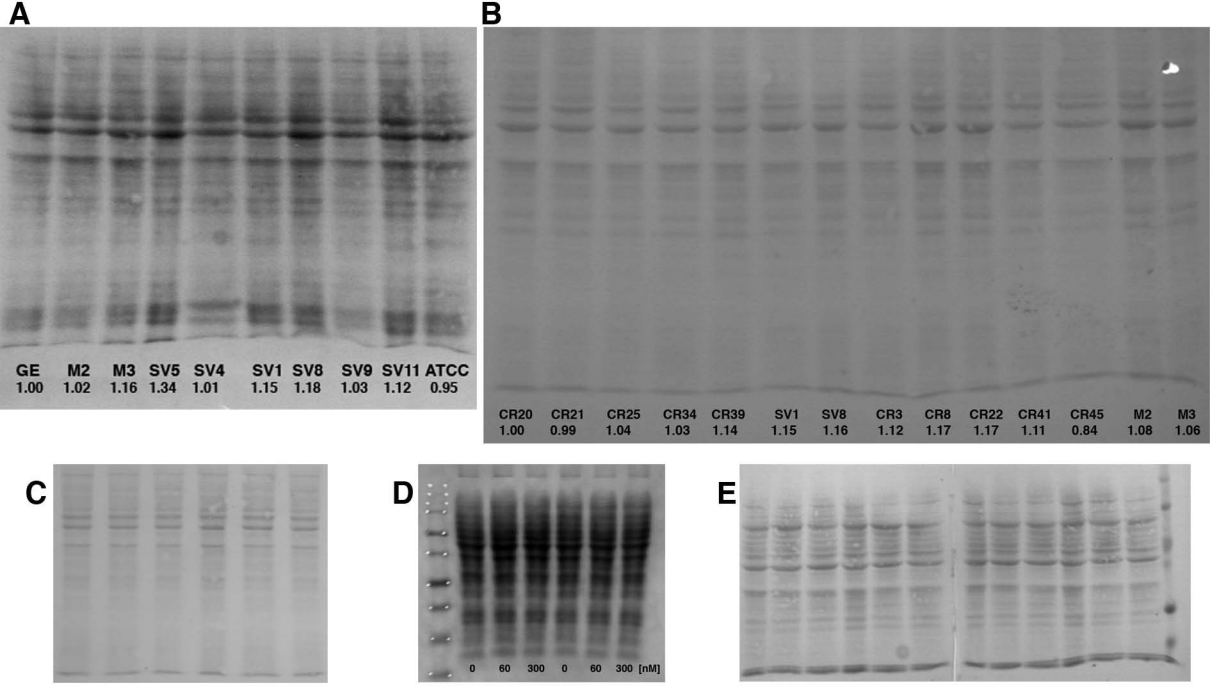
Supplemental Fig. S1 Cell numbers and OD 540 nm values from the MTT assay are highly correlated. Met-5A cell clones M3 and SV8 were seeded at different densities (1000 – 5000 cells per well) and the MTT assay was performed 3 days later. A linear relationship existed between the MTT signal and cell numbers of up to 3000 cells/well. When cells were seeded at high density, reaching confluency, before performing the MTT assay, the MTT signal reached a plateau and did not accurately reflect the initial cell number. Thus, the number of cells/well was adapted for each type of experiment to verify that confluency was not reached even under control conditions (no treatment).

Supplemental Fig. S2 Increased expression of calretinin in MeT-5A-GE cells stably transfected with the plasmid pCMV-Tag-NEO. Western blot for calretinin (top) and the corresponding Ponceau S-stained membrane (bottom) used for normalization. MeT-5A-GE cells stably transfected with the plasmid pCMV-Tag-NEO before isolating individual clones were compared to the parental MeT-5A-GE cells. The calretinin Western blot signal of transfected cells that survived the G418 selection (post) was approximately 2-fold higher than in the parental MeT-5A-GE cells (pre), indicating that proteins expressed by the plasmid (Tag and tag) upregulate calretinin expression. Right lane: purified recombinant calretinin (CR) used as a control.

Supplemental Fig. S3 Ponceau S-stained membranes used for the normalization of the Western blot signals. For normalization of the Western blots, densitometric analysis of the Ponceau S-stained membranes using the GeneTools Software (Syngene, Cambridge, UK) was performed. A) Membrane used for the quantification of results shown in Fig. 2A and 3B. B) Membrane used for the quantification of results shown in Fig. 4A. In A) and B), the values below the clone names represent relative values (normalization factors), the left lanes on each gel were defined as 1.00. C) Membrane used for the quantification of results shown in Fig. 5A (siRNA experiments). D) Membrane used for the quantification of results shown in Fig. 5A (antisense oligo experiments). E) Membrane used for the quantification of results shown in Fig. 6B (PI3K inhibitor PI103); membrane used to quantify pAKT (left 6 lanes) and for total AKT (right 6 lanes).


1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
OD 540
M3
SV8
0
1000
2000
3000
4000
5000
cells/well


pre
post
CR


A
GE M2 M3 SV5 SV4 SV1 SV8 SV9 SV11 ATCC
1.00 1.02 1.16 1.34 1.01 1.15 1.18 1.03 1.12 0.95
B
CR20 CR21 CR25 CR34 CR39 SV1 SV8 CR3 CR8 CR22 CR41 CR45 M2 M3
1.00 0.99 1.04 1.03 1.14 1.15 1.16 1.12 1.17 1.17 1.11 0.84 1.08 1.06
C
D
0 60 300 0 60 300 [nM]
E