Supplemental Figure S1 Cuticle phenotypes of lwr mutant embryos

(A) Cuticle phenotypes from wildtype embryos or those from females carrying lwr^{118} homozygous mutant germline clones (hereafter called lwr mutant embryos). Different classes of phenotype are observed for the lwr mutant embryos as shown, wildtype cuticles are also observed (not shown).

(B) Graph shows quantification of the different classes of cuticles following counts of 400 embryos. Approximately 50% of the lwr mutant embryos have wildtype cuticles, consistent with paternal rescue of the mutant phenotype as described previously (Epps and Tanda 1998). The presence of a posterior hole in some embryos and puckered phenotype of others are characteristics of misregulation of Dpp signaling (Martin-Blanco et al. 2000; Podos et al. 2001).
Supplemental Figure S2 sog and dpp expression in the lwr mutant embryos
Lateral views of cellularised wildtype and lwr mutant embryos stained with sog and dpp RNA probes. Both expression patterns are essentially wildtype in the lwr mutants.
Supplemental Figure S3 Quantification of the number of Krüppel positive amnioserosa cells in transgenic embryos

(A) Detection of Kr protein in embryos which are either wildtype, *tub*-GAL4; pUAS-Med or *tub*-GAL4; pUAS-GFP-Med, as labelled. Graph shows quantification of Kr positive amnioserosa cells, n=8, error bars are s.e.m., *P<0.05.

(B) As in (A), except the embryos are heterozygous for Ub:Med or Ub:Med-SUMO transgenes.
Supplemental Figure S4 Quantification of Race expression in additional transgenic lines

(A) Race expression in wildtype embryos and 2 independent lines carrying the Med-SUMO transgene. Embryos are at the onset of gastrulation with dorsal up. The number of expressing cells in the middle of the embryo was counted for 15 embryos at the correct stage and the averages are plotted in the graph. Error bars denote s.e.m., n=15

(B) As in (A), except that lines 1 and 2 have independent insertion sites for the Med and MedABC transgenes.
Supplemental Figure S5 Comparison of Med and MedABC transcriptional activity

The transcriptional activities of Med and MedABC were analysed following transfection of the appropriate Med expression plasmid into 293 cells with Mad, Tkv-QD and the SBE₄-luciferase reporter gene (Zawel et al. 1998). The fold change in activation of the luciferase reporter by MedABC compared to wildtype Med was calculated in the presence and absence of Gam1 and plotted on the graph. Gam1 inhibits the SUMO pathway by targeting the E1 enzyme (Boggio et al. 2004). MedABC has higher transcriptional activity than Med only when the SUMO pathway is active, suggesting that the MedABC mutations exclusively affect SUMOylation.
Supplemental Figure S6 Photobleaching experiments

(A, B) MDA-MB468 cells were transfected with Tkv-QD, Mad and either GFP-Med or GFP–MedABC. The complete set of recovery images captured over 200 sec is shown for GFP-Med (A) and GFP-MedABC (B) following photobleaching of the nucleus. The prebleach image is shown in Fig. 7.
References