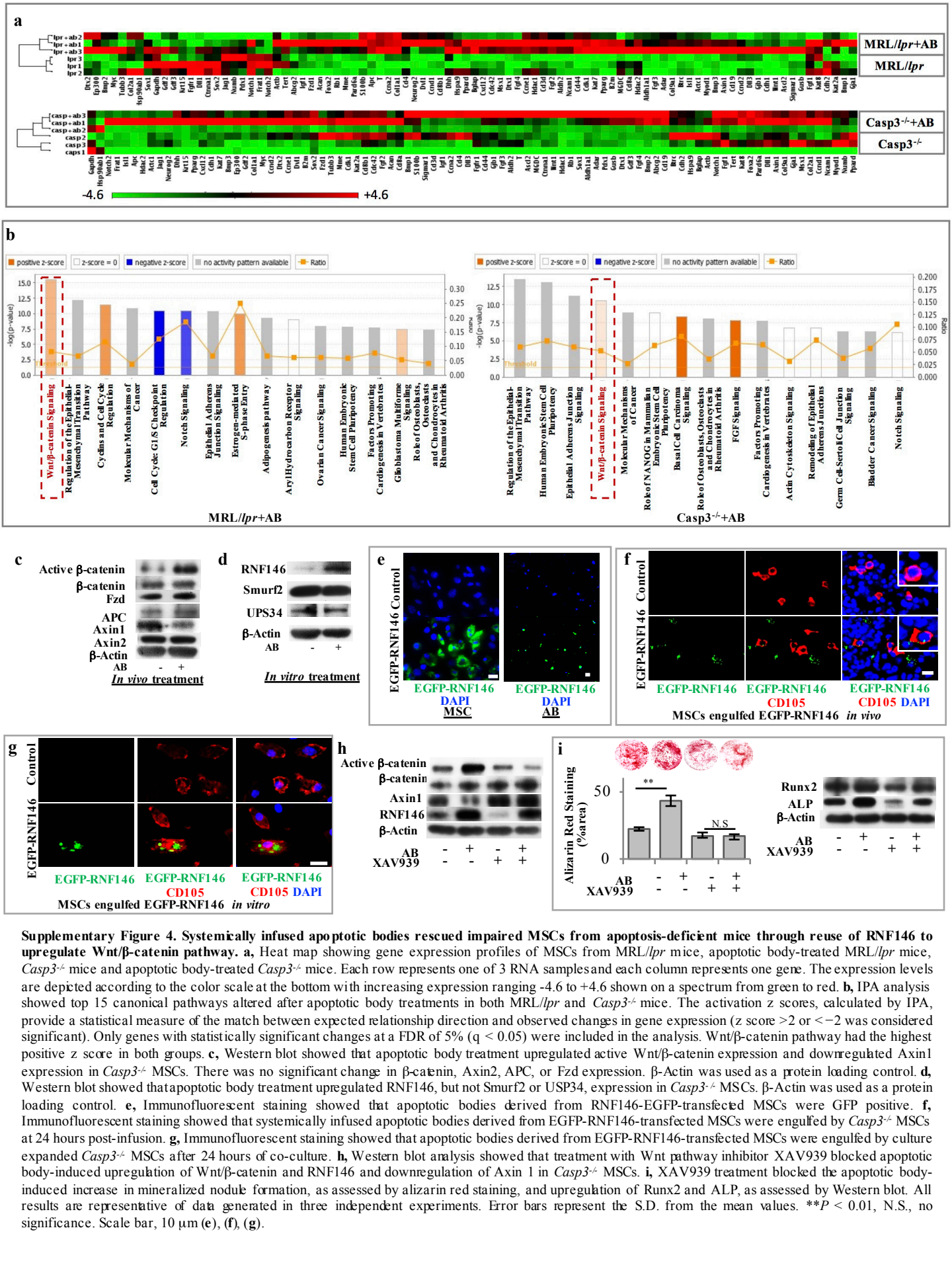

Supplementary Figure 4. Systemically infused apoptotic bodies rescued impaired MSCs from apoptosis-deficient mice through reuse of RNF146 to upregulate Wnt/β-catenin pathway. a, Heat map showing gene expression profiles of MSCs from MRL/*lpr* mice, apoptotic body-treated MRL/*lpr* mice, *Casp3*$^{-/-}$ mice and apoptotic body-treated *Casp3*$^{-/-}$ mice. Each row represents one of 3 RNA samples and each column represents one gene. The expression levels are depicted according to the color scale at the bottom with increasing expression ranging -4.6 to +4.6 shown on a spectrum from green to red. **b,** IPA analysis showed top 15 canonical pathways altered after apoptotic body treatments in both MRL/*lpr* and *Casp3*$^{-/-}$ mice. The activation z scores, calculated by IPA, provide a statistical measure of the match between expected relationship direction and observed changes in gene expression (z score >2 or <−2 was considered significant). Only genes with statistically significant changes at a FDR of 5% ($q < 0.05$) were included in the analysis. Wnt/β-catenin pathway had the highest positive z score in both groups. **c,** Western blot showed that apoptotic body treatment upregulated active Wnt/β-catenin expression and downregulated Axin1 expression in *Casp3*$^{-/-}$ MSCs. There was no significant change in β-catenin, Axin2, APC, or Fzd expression. β-Actin was used as a protein loading control. **d,** Western blot showed that apoptotic body treatment upregulated RNF146, but not Smurf2 or USP34, expression in *Casp3*$^{-/-}$ MSCs. β-Actin was used as a protein loading control. **e,** Immunofluorescent staining showed that apoptotic bodies derived from RNF146-EGFP-transfected MSCs were GFP positive. **f,** Immunofluorescent staining showed that systemically infused apoptotic bodies derived from EGFP-RNF146-transfected MSCs were engulfed by *Casp3*$^{-/-}$ MSCs at 24 hours post-infusion. **g,** Immunofluorescent staining showed that apoptotic bodies derived from EGFP-RNF146-transfected MSCs were engulfed by culture expanded *Casp3*$^{-/-}$ MSCs after 24 hours of co-culture. **h,** Western blot analysis showed that treatment with Wnt pathway inhibitor XAV939 blocked apoptotic body-induced upregulation of Wnt/β-catenin and RNF146 and downregulation of Axin 1 in *Casp3*$^{-/-}$ MSCs. **i,** XAV939 treatment blocked the apoptotic body-induced increase in mineralized nodule formation, as assessed by alizarin red staining, and upregulation of Runx2 and ALP, as assessed by Western blot. All results are representative of data generated in three independent experiments. Error bars represent the S.D. from the mean values. $^{**}P < 0.01$, N.S., no significance. Scale bar, 10 μm (**e**), (**f**), (**g**).