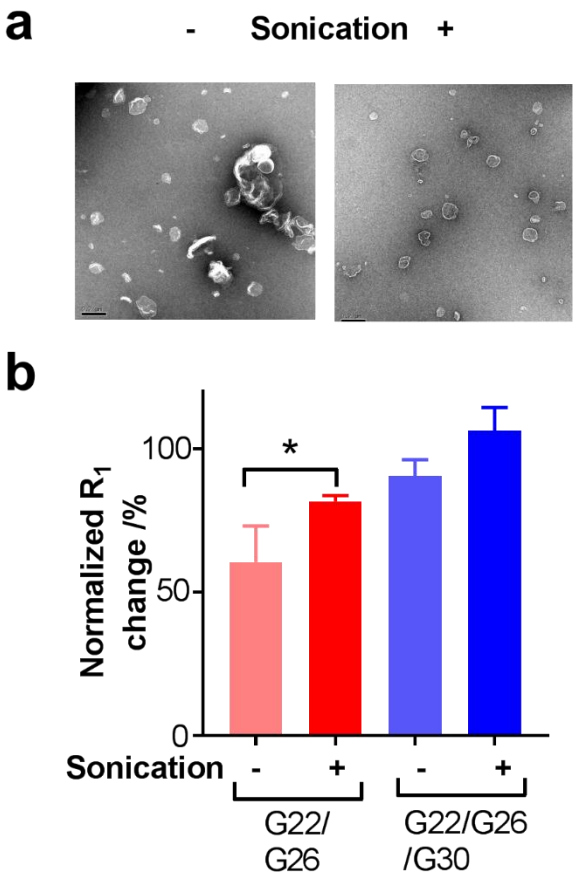

Figure S1. The effect of sonication on the size of paramagnetic liposomes reconstituted with MscL nanovalve

A: TEM shows that the size of liposomes reconstituted with labelled G22/G26/G30 MscL before sonication (left) varies and liposomal clusters are frequently seen. After sonication (right) their size is more uniform with an average diameter of 100 nm. The majority of liposomes had a diameter below 150 nm. B: R_1 measurement shows that the percentage signal change upon a pH decrease from 7.4 to 5.5 is significantly higher after the sonication of the liposomes. *: $p < 0.05$, $n = 4$ and 3 for G22/G26 and G22/G26/G30 proteoliposomes, respectively, student *t* test.

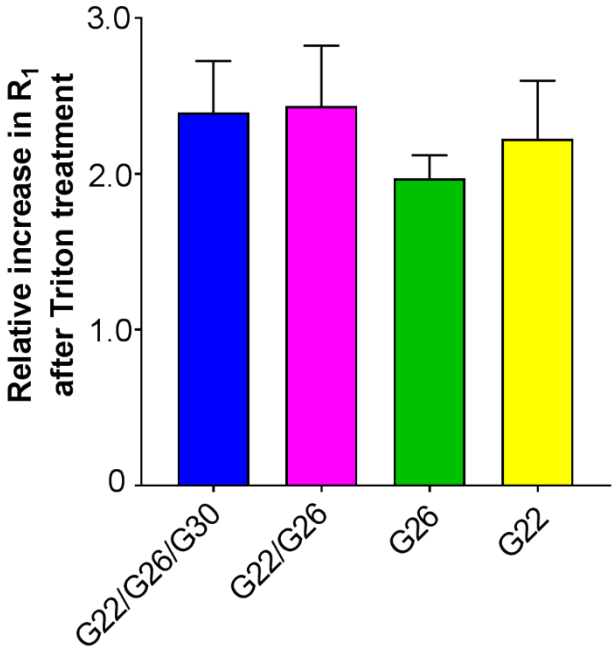

Figure S2: Loading of proteoliposomes used in experiments shown in Figure 2.

Loading of Gd-DOTA inside proteoliposomes is expressed as relative increase in R_1 relaxation rate after 0.5 % Triton X-100 treatment to completely lyse the vesicles. *n* equals 5, 5, 3 and 4 for G22/G26/G30, G22/G26, G26C and G22C proteoliposomes, respectively.

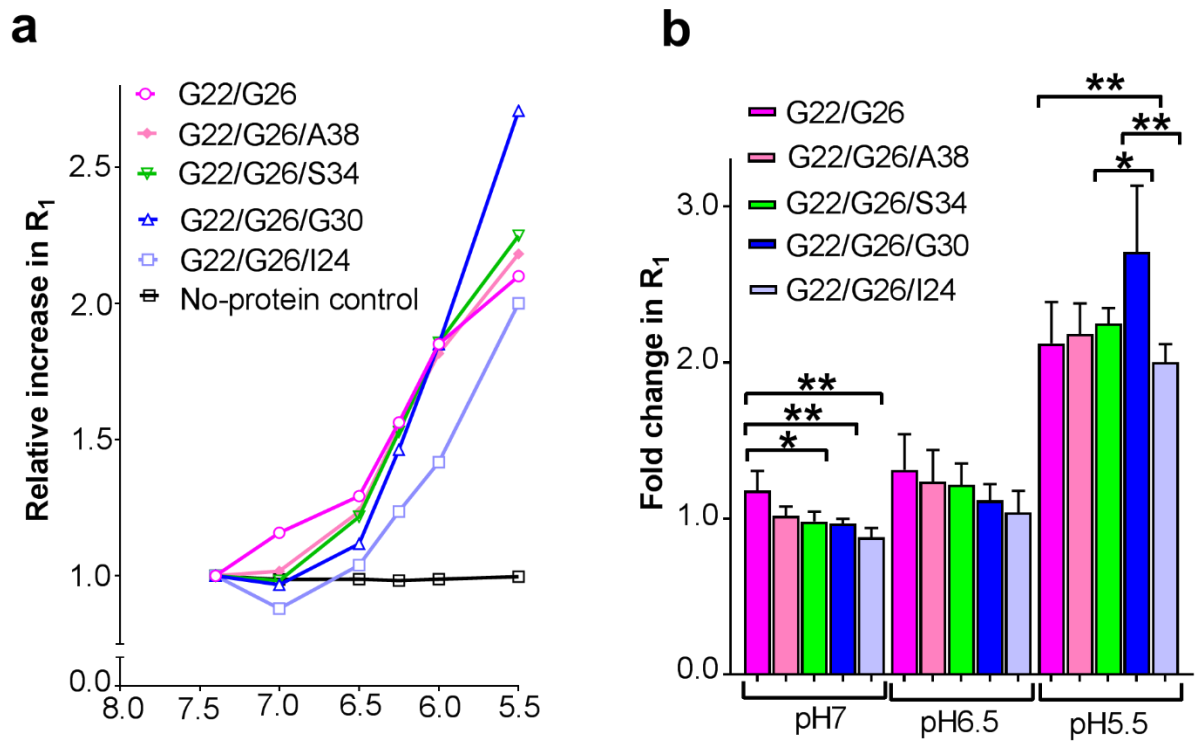

Figure S3: Increase of R_1 relaxation rates upon pH-stimulus of all double and triple labeled MscL tested.

a. The R_1 relaxation rate of proteoliposomes reconstituted with G22/G26 (magenta circle, *n* = 7), G22/G26/A38 (pink diamond, *n* = 3), G22/G26/S34 (green triangle, n=3), G22/G26/G30 (blue triangle, *n* = 7) and G22/G26/I24 (light blue square, *n* = 3) was plotted against decreasing pH. No-protein control group was shown as black squares (*n* = 10). **b**. R_1 of proteoliposomes reconstituted with all mutants tested was compared at pH 7, 6.5 and 5.5. "*", $p < 0.05$, "**"$p < 0.01$, student *t* test.