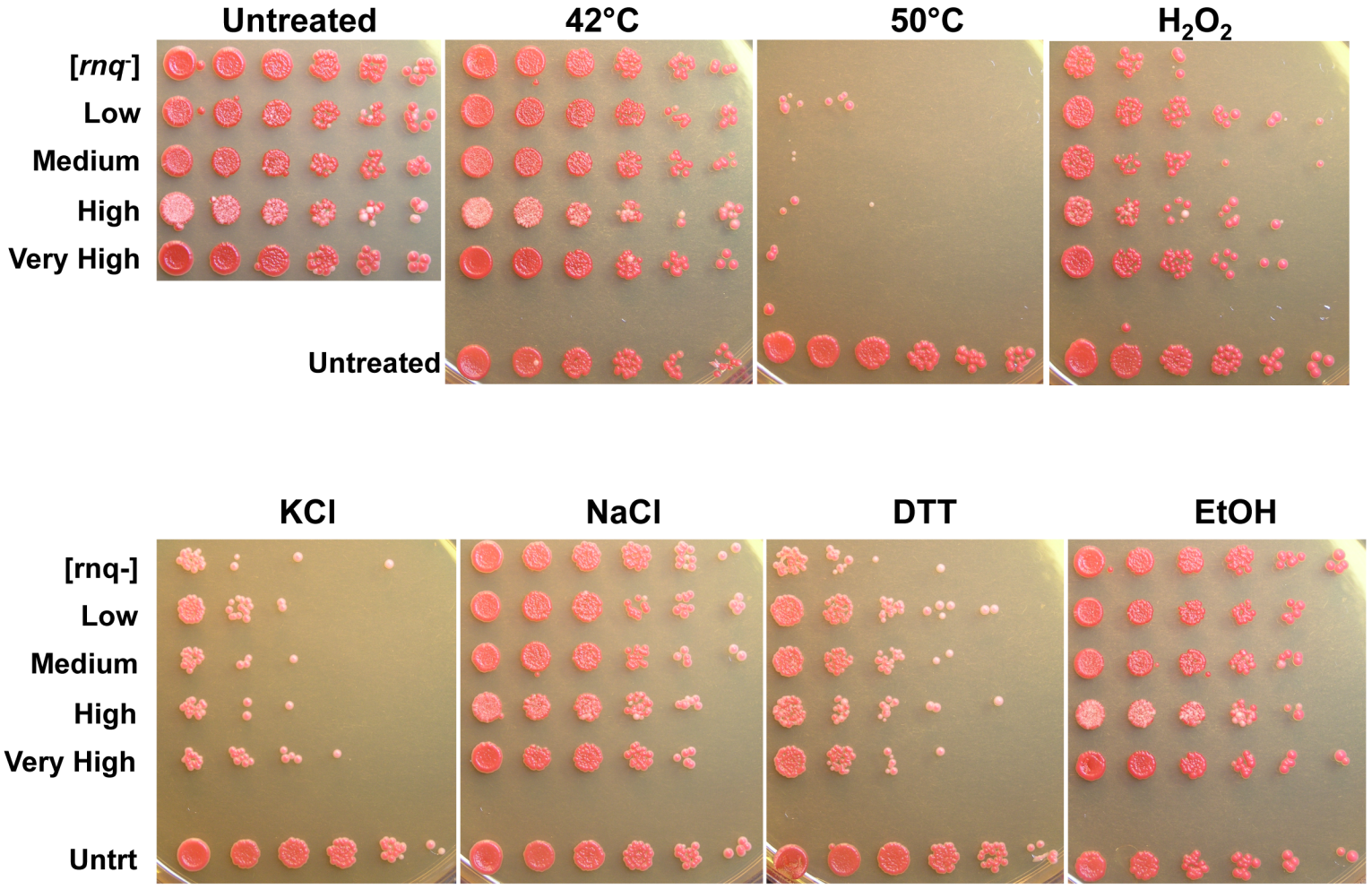
Supplemental Figure 1



Supplemental Figure 1. Stressors exert varying degrees of toxicity. 74-D694 yeast harboring the low, medium, high or very high [*RNQ*+] variants or [*rnq*-] cells were normalized by OD600 and subjected to indicated environmental stressors as described in Materials and Methods and Supplemental Table 1. Cells were serially diluted 5x, spotted on solid YPD rich media and grown for 3 days at 30°C with a control untreated strain containing low [*RNQ*+] that was also spotted on each plate.

Supplemental Figure 2

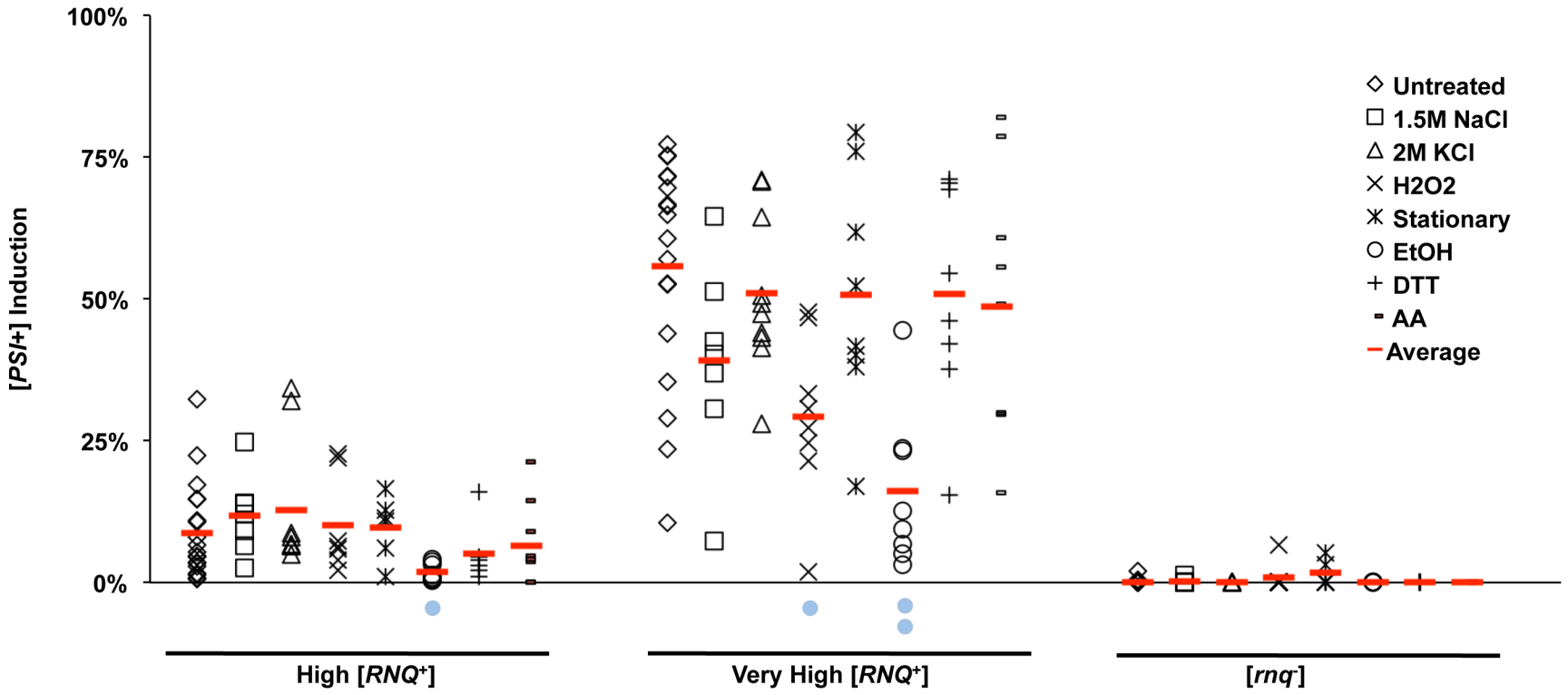
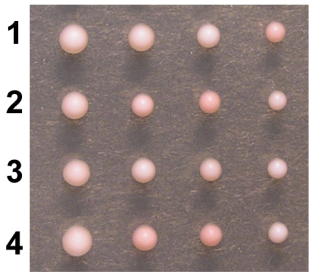


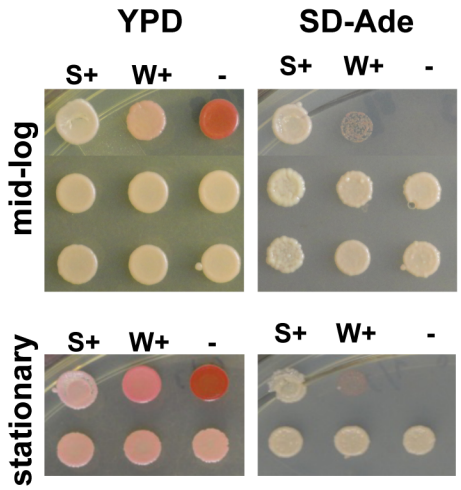
Figure 2. Extracellular environment influences [*PSI*+] induction and variant formation in a [*RNQ*+] variant-dependent manner. 74-D694 yeast cells containing either the low or medium [*RNQ*+] variants were treated with or without environmental stress and scored for [*PSI*+] status using the [*PSI*+] induction assay, as described in Materials and Methods. At least 1,500 colonies from at least 3 independent experiments were scored for each condition. Red lines denote the average of all experiments. Two blue dots below a condition indicate $p<0.0001$ and one blue dot below a condition indicates $p<0.05$ statistically significant difference from the untreated cells with the same [*RNQ*+] variant when analyzed by two-tailed Student's t-test. The number of colonies scored for each of the stress conditions ranged from 40 to 669 with an average number of colonies scored of ~300. Conditions that did not alter [PSI+] variants as compared to untreated controls were excluded from the chart. Percentage of total [*PSI*+] variants for each condition is plotted.

Supplemental Figure 3



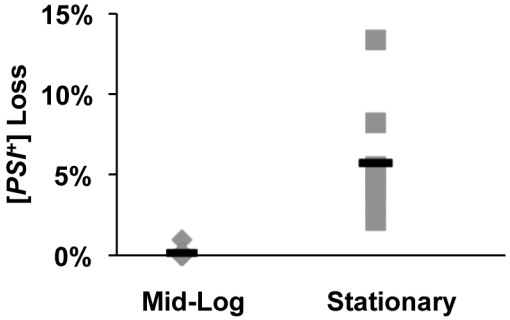
Supplemental Figure 3. Color phenotype corresponds with the presence of the dominantly inherited [*PSI+*] prion. (A) Cells harboring [*PSI+*] that spontaneously formed in stationary phase were mated to [*psi-*] cells and resultant tetrads were dissected and plated. This selection of tetrads represents 4 of more than 40 dissected and analyzed.

Supplemental Figure 4



Supplemental Figure 4. Incubation in stationary phase induces strong [*PSI*+]. Yeast harboring the different [*RNQ*+] variants were incubated either overnight at 30°C (log-phase) or 7 days at 30°C (stationary phase). Diluted cells were spread on YPD or SD-Ade plates and grown at 30°C for 3 or 8 days, respectively. Colonies that were adenine prototrophs were picked and spotted on YPD, SD-Ade and YPD + 3mM GdnHCl solid media and scored for curability and strength of [*PSI*+] by growth on SD-Ade. A representative spotting is shown.

Supplemental Figure 5



Supplemental Figure 5. Incubation in stationary phase increases the loss of weak [*PSI*+]. Yeast harboring an established weak [*PSI*+] variant were incubated either overnight at 30°C (O/N) or 7 days at 30°C (stationary phase). Diluted cells were spread on YPD plates and grown at 30°C for 5 days. Colonies that were red or contained red sectors were scored as [*psi*-] and [*PSI*+] loss was calculated as the number of [*psi*-] colonies divided by the total number of colonies. Three experiments with 3 cultures per experiment were conducted and a Student's t-test was performed to define significant differences between conditions ($p<0.0003$).

Supplemental Table 1. Summary of environmental stress conditions tested for their impact on [*PSI*$^+$] formation.

Stress	Starting OD_{600}=	Conditions	Dilution plated
Untreated	1.00	30°C standard growth	1:8000
1.5M NaCl	0.25	20 hours in SD-Ura + 1.5M NaCl at 30°C	1:8000
2M KCl	0.25	20 hours in SD-Ura + 2M KCl at 30°C	1:4000
42°C	0.50	37°C for 30', 42°C for 40'	1:8000
50°C	0.50	37°C for 30', 50°C for 40'	1:10
10mM H_2O_2	1.00	SD-Ura + 10mM H2O2 for 60' at 30°C	1:100
12% EtOH	0.50	SD-Ura + 12% EtOH for 30' at 30°C	1:1500
Stationary	1.00	0.5ml o/n culture into 25ml YPD for 7d at 30°C	1:20,000
AA deprivation	1.00	Spin down overnight culture normalized to OD=1.0. Wash 1x with H_2O. Resuspend to OD=1 in SD-Ura-His. Put back at 30°C for 3hrs.	1:8000
12mM DTT	0.25	20 hours in SD-Ura + 12mM DTT at 30°C	1:500