



# Cell-Signalling in Streptococcus Pneumoniae (SPN)

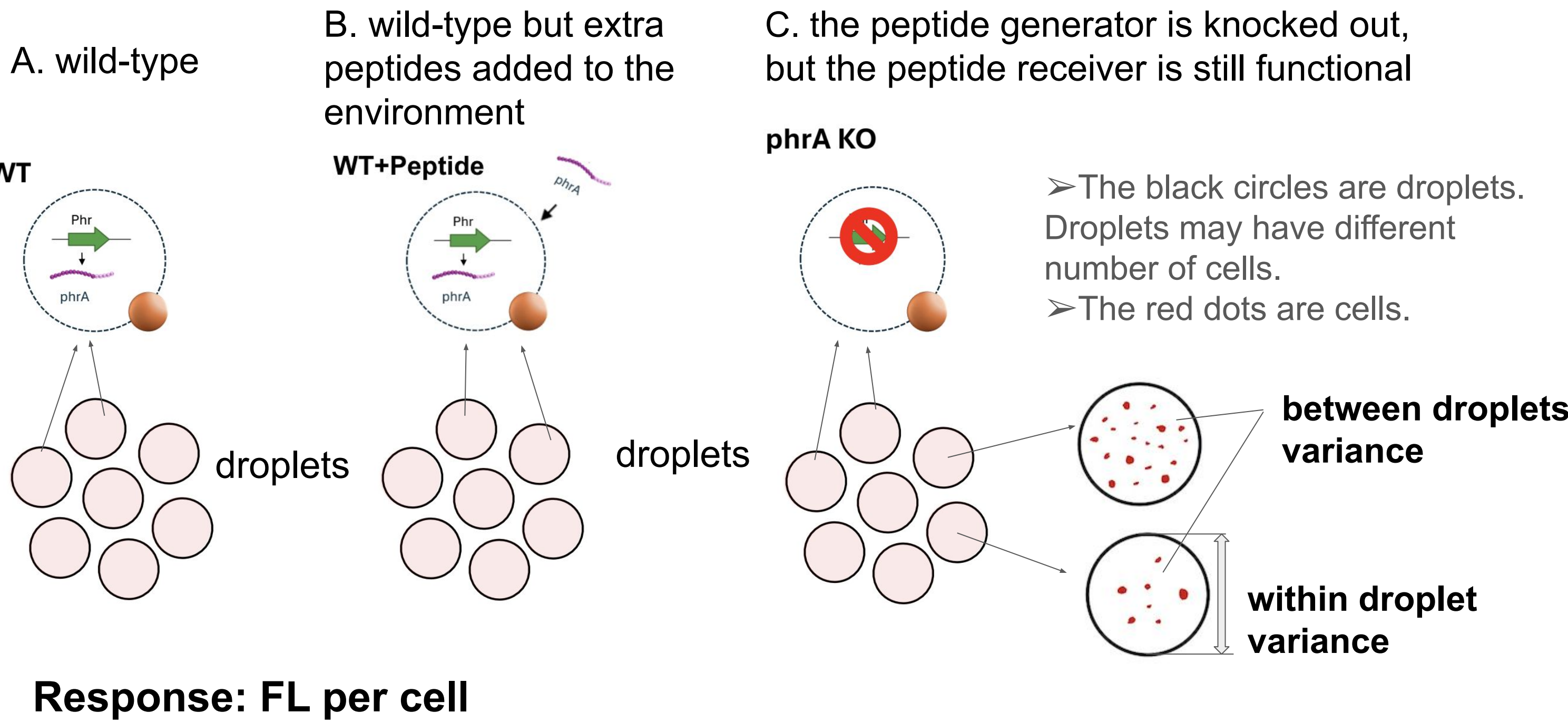
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## Background

- *Spn* is the leading cause of pneumonia mortality globally, killing more than 300,000 children under 5 years old worldwide annually
- *Spn* cells are inserted in microdroplets
- How cells communicate → use the **peptide** produced by PhrA induction
- How we measure communication → **fluorescence intensity (FL)**
  - more peptides → more communication → greater FL
- Research Objective
  - **Investigate cell communication under 3 experiment conditions**

## Data

Experimental Conditions – approximately 35-40 droplets @4 hr



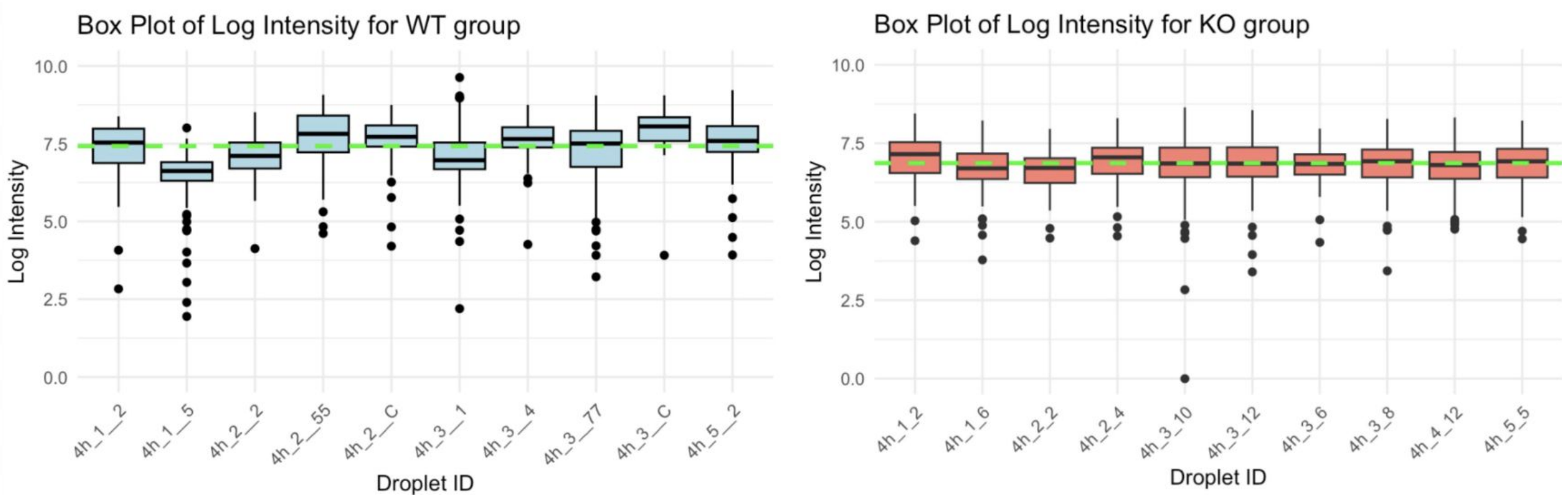
## Method

### Mixed effect model

- Model that accounts for both fixed effects (systematic, population-level variability) and random effects (individual-level variability)
- model = lmer(response ~ fixed effect + (1 | random effect), data = data)
- Intraclass Correlation Coefficient (ICC) measures the proportion of total variance in the response variable that is attributable to between unit (droplet) variation

$$\text{ICC} = \frac{\sigma^2_{\text{between}}}{\sigma^2_{\text{between}} + \sigma^2_{\text{within}}}$$

## Results & Analysis



- **Random selection** of 10 droplets
- Mean of WT (greenline) higher than KO
- WT group has more variation **both between and within droplet** and has more extreme outliers

Table 1: Comparison of KO vs WT @ 4 hrs

Exp	Measure	Mixed Model		Fixed Effects	
		KO	WT	KO	WT
Exp #1	Mean (KO)	6.752		6.764	
	<b>Group Effect (WT)</b>	<b>0.582</b>		<b>0.552</b>	
	t-value (group)	8.554		29.61	
	ICC	10.35%		NA	
Exp #2	Mean (KO)	7.10398		6.82107	
	<b>Group Effect (WT)</b>	<b>0.43544</b>		<b>0.52545</b>	
	t-value (group)	5.401		32.27	
	ICC	9.55%		NA	
Exp #3	Mean (KO)	6.722		6.873	
	<b>Group Effect (WT)</b>	<b>0.498</b>		<b>0.546</b>	
	t-value (group)	22.04		32.74	
	ICC	12.06%		NA	

- **More active communication in WT than KO**
  - mean FL WT > mean FL KO
- **Between-droplet variability is significant**
  - ICC significantly > 0
- **t-values differ** significantly between two models
- **Fixed-effect** models yield much **larger** t-values
- Mixed model accounts for droplet-level variability
  - more conservative significance

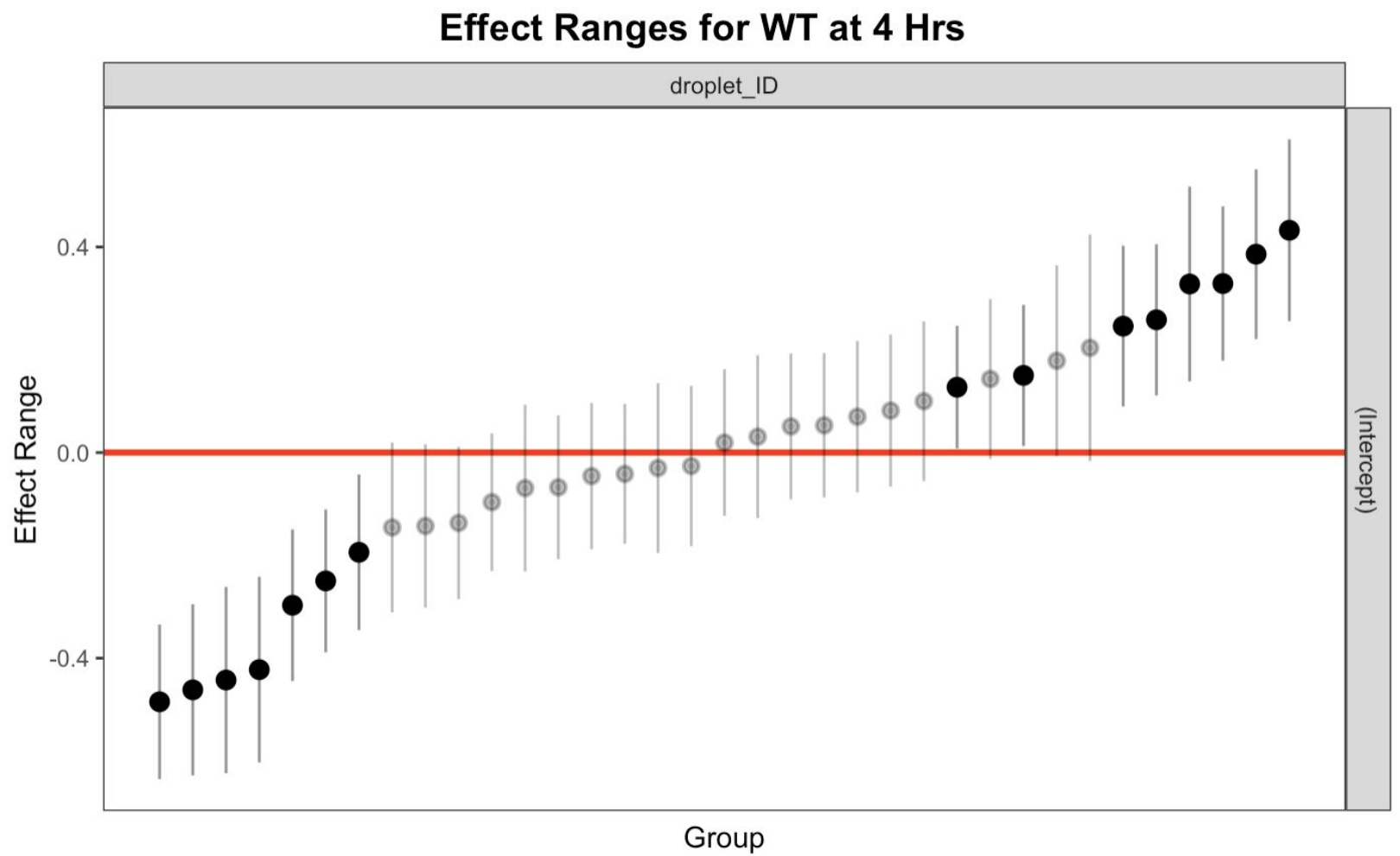
## Conclusion

### Biological insights

- WT cells show **stronger fluorescence** than KO
- WT+peptide further increases fluorescence Intensity
- **Between-droplet variability** is consistently significant across experiments

### Modeling Insights

- Fixed-effects models lead to inflated t-values and potentially **overstated** significance by ignoring between-droplet variability
- **Mixed effect model is more suitable for this experiment**



- Figure shows each droplet's 95% CI for the deviation in mean FL from the overall WT average
- There is **heterogeneity** among WT droplets in their mean FL under the 4-hour condition

Table 2: Comparison of WT vs WT+Peptide @ 4 hrs

Exp	Measure	Mixed Model		Fixed Effects	
Exp #1	Intercept (WT)	7.006		6.964	
	<b>Group Effect (+ peptide)</b>	<b>0.391</b>		<b>0.426</b>	
	t-value (group)	14.30		22.86	
	ICC	10.90%		NA	
Exp #2	Intercept (WT)	6.900		6.821	
	<b>Group Effect (+ peptide)</b>	<b>0.423</b>		<b>0.525</b>	
	t-value (group)	16.99		32.27	
	ICC	7.05%		NA	
Exp #3	Intercept (WT)	7.191		7.092	
	<b>Group Effect (+ peptide)</b>	<b>0.244</b>		<b>0.383</b>	
	t-value (group)	8.41		18.31	
	ICC	12.38%		NA	

- t-values of group effect range from 8.41 to 32.27, all significant with **p <.001** → **strong statistical support** for group effect
- **More active communication in WT+Peptide**
  - mean FL WT+Peptide > mean FL WT
- ICC shows **significant variability across droplets**

## References

- **Clark, M.** (2020). *Random Intercepts — Mixed Models with R*. Retrieved from m-clark.github.io
- **Mudge, L.** (n.d.). *Mixed Effects Models*. Retrieved from lmudge13.github.io
- **Aggarwal, S. D., Yesilkaya, H., Dawid, S., & Hiller, N. L.** (2020). *The pneumococcal social network*. PLOS Pathogens, 16(10), e1008931. <https://doi.org/10.1371/journal.ppat.1008931>