Molecular response to heat stress and lipopolysaccharide in chicken macrophage-like cell line

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Heat Stress in Changing Climates

- **Ambient temperatures > 26°C**
- **Relative humidity > 40%**
  - Problem of tropical climates – „modern” vs. indigenous breeds
  - Climate change – more frequent heat waves in moderate climates

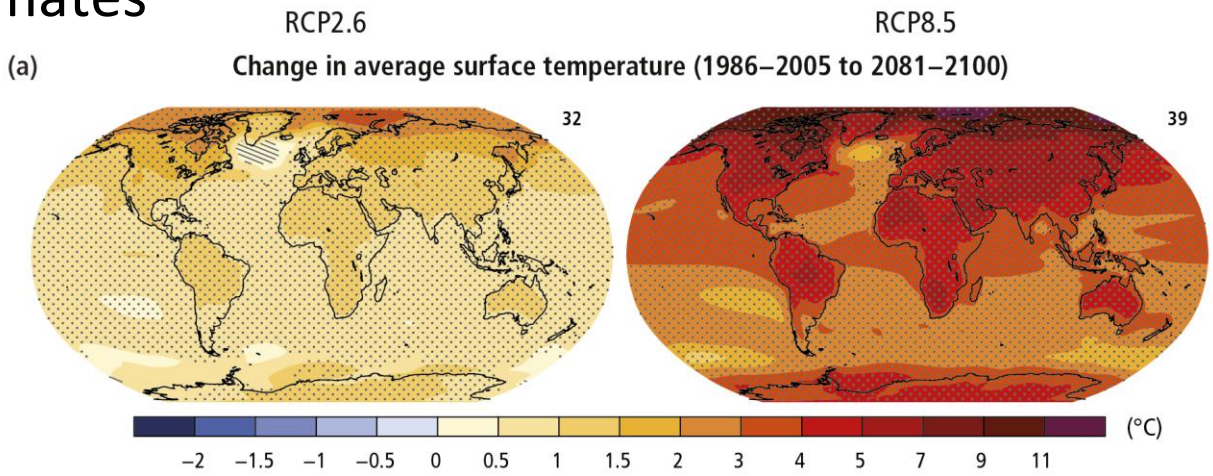
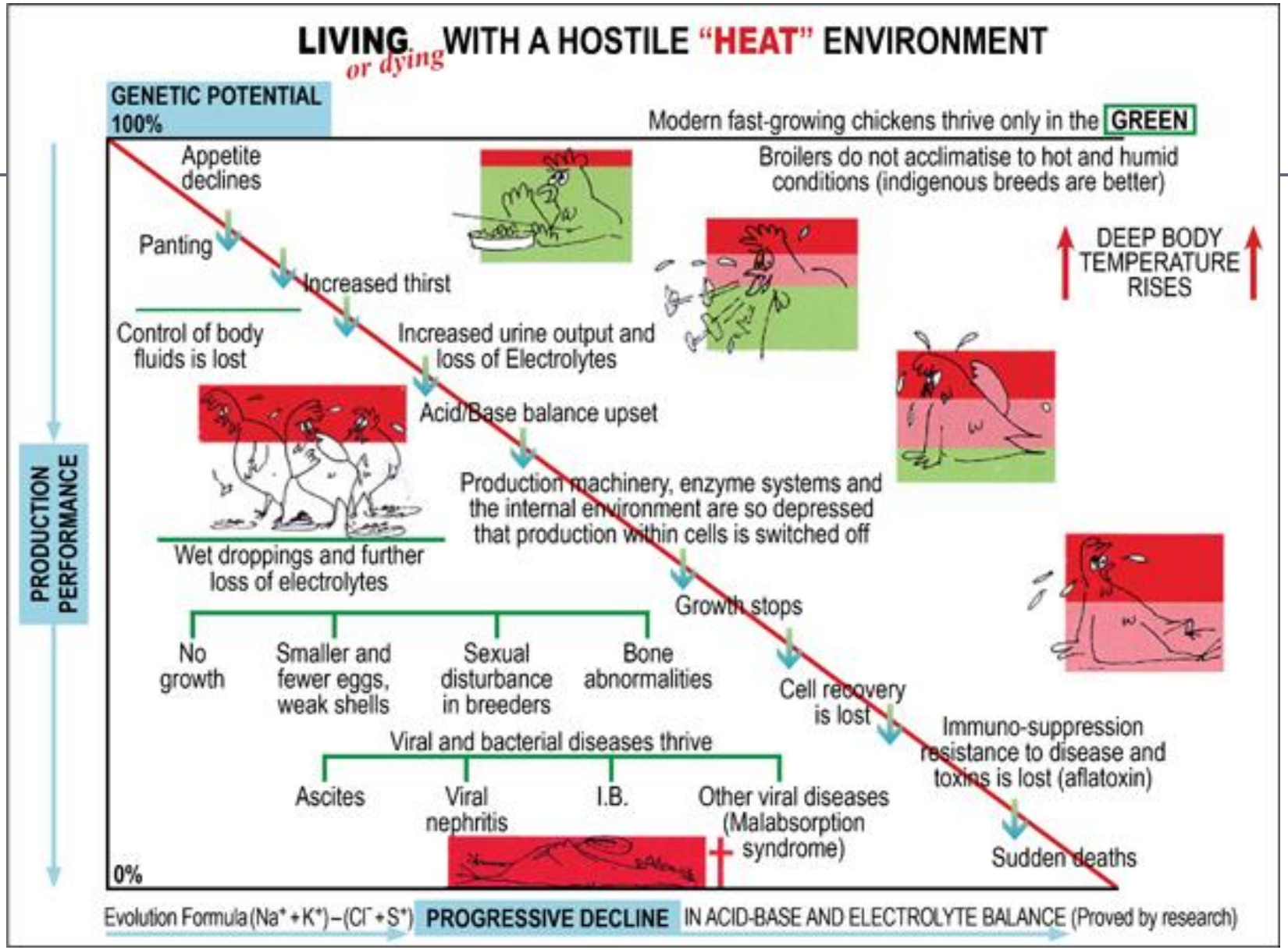


Fig. 1 Geographical pattern of surface warming for late 21st century (2090-2099) relative to 1986-2005. Source: IPCC Fourth Assessment Report: Climate Change 2014

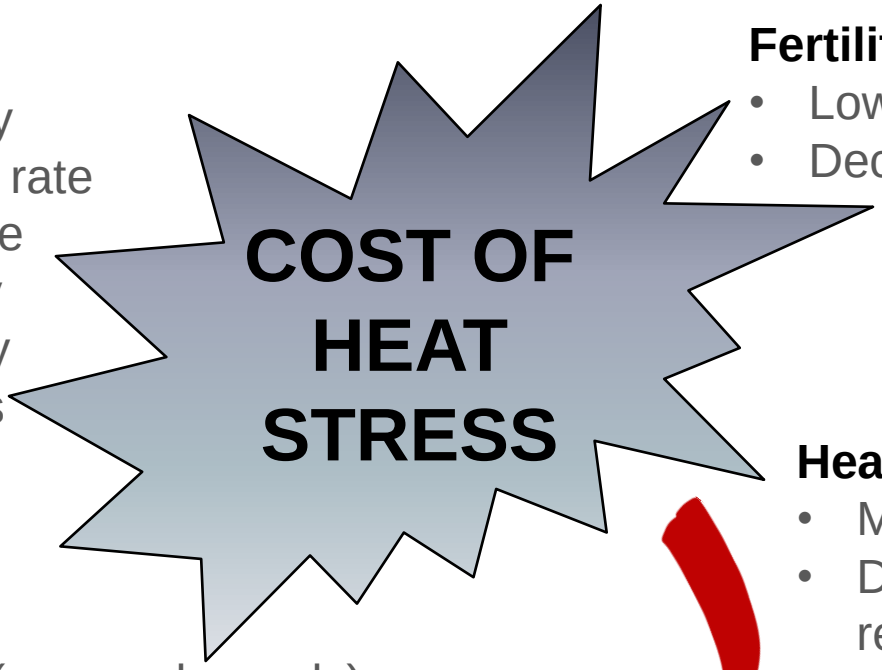


http://www.heatstress.info/HeatStressExplained/HowtorecogniseHeatStressinpoultry.aspx

Cost of Heat Stress in Poultry

Productivity losses
- Increased mortality
- Decreased growth rate
- Depressed appetite
- Lower shell quality
- Lower meat quality
- Loss of electrolites

Investments
- Ventilation
- Shades
- Cooling systems (e.g. cooler pads)



Fertility losses
- Lower egg production
- Decreased fertility of males

Health decline
- Metabolic disorders
- Decreased disease resistance
- Systemic inflammation

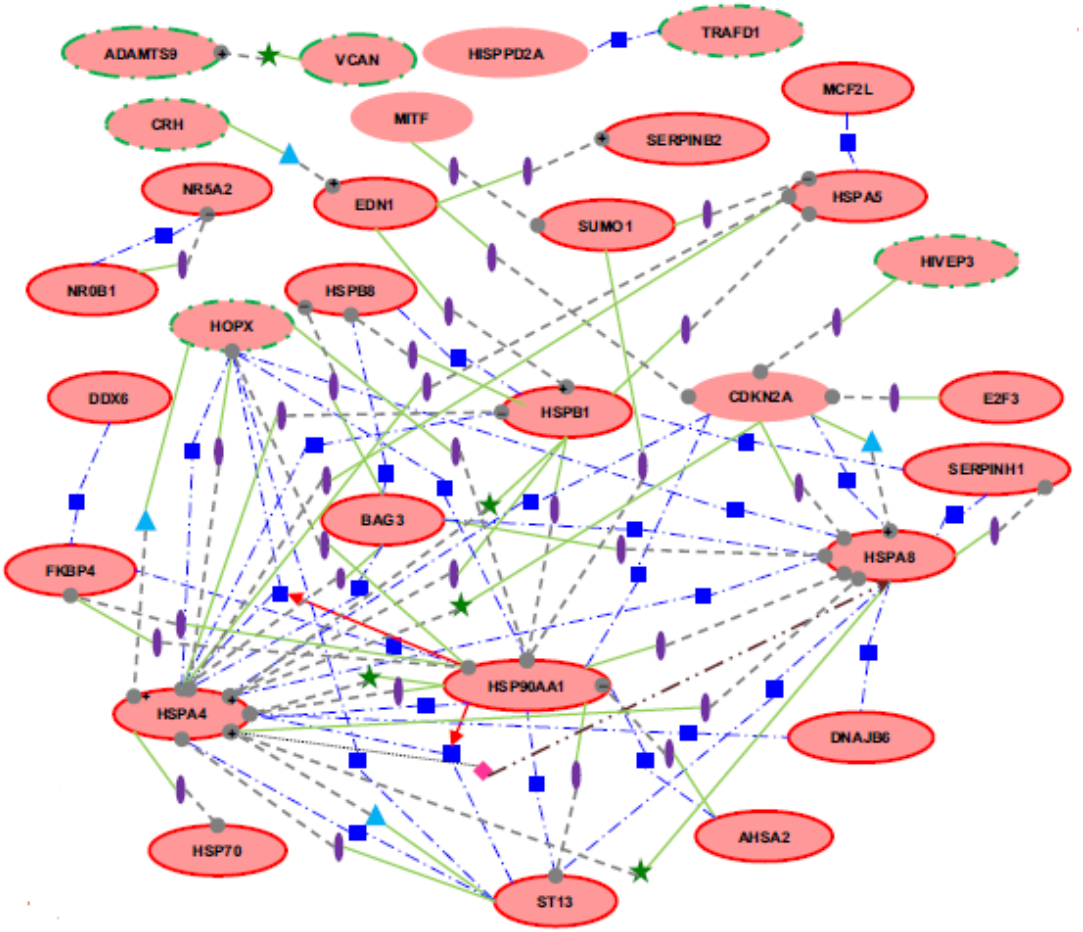
$125-165 million per year in USA

(St-Pierre et al., 2003)

Adapted from http://www.heatstress.info/

Whole-genome molecular responses to heat stress in chickens

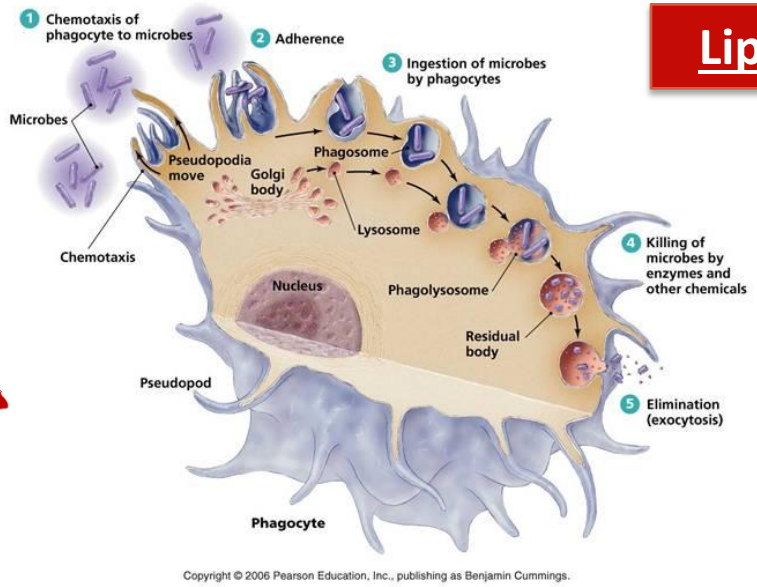
- **Brain**
  - Li et al., 2011
- **Liver**
  - Coble et al., 2014
  - Luo et al., 2014
- **Muscle**
  - Li et al., 2011
  - Luo et al., 2014
- **Testes**
  - Wang et al., 2014
- **LMH cell line**
  - Sun et al., 2015



Heat stress and endotoxemia in chicken immune cells (HD11)

HEAT STRESS

Lipopolysaccharide (LPS)



EXPERIMENTAL OUTLINE

HS, LPS

2h 4h 6h 10h

45°C **41.5°C**

EAAP, Warsaw, Aug 31 2015

* The experiment was replicated three times

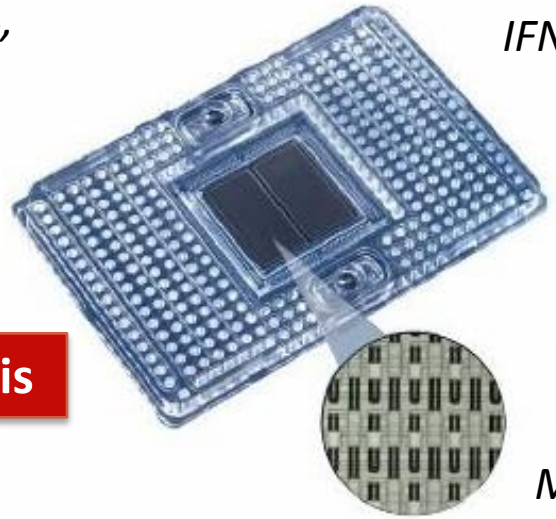
Gene expression study

Heat shock proteins & factors

HSP25, HSPA2, HSPA14, HSPB8, HSP90AAI, HSPH1, HSF2, HSF4, HSF5, DNAJA4

Immune response

IL1B, IL8, CD40, IL12B, IL18, LITAF, IFNB, IFNG, iNOS, CCL4, CCL5



Stress response & apoptosis

SERPINH1, BAG3, RB1CC1, UBB, CIRBP, TP53, CASP1, CASP3, CASP7, CASP9, CASP8

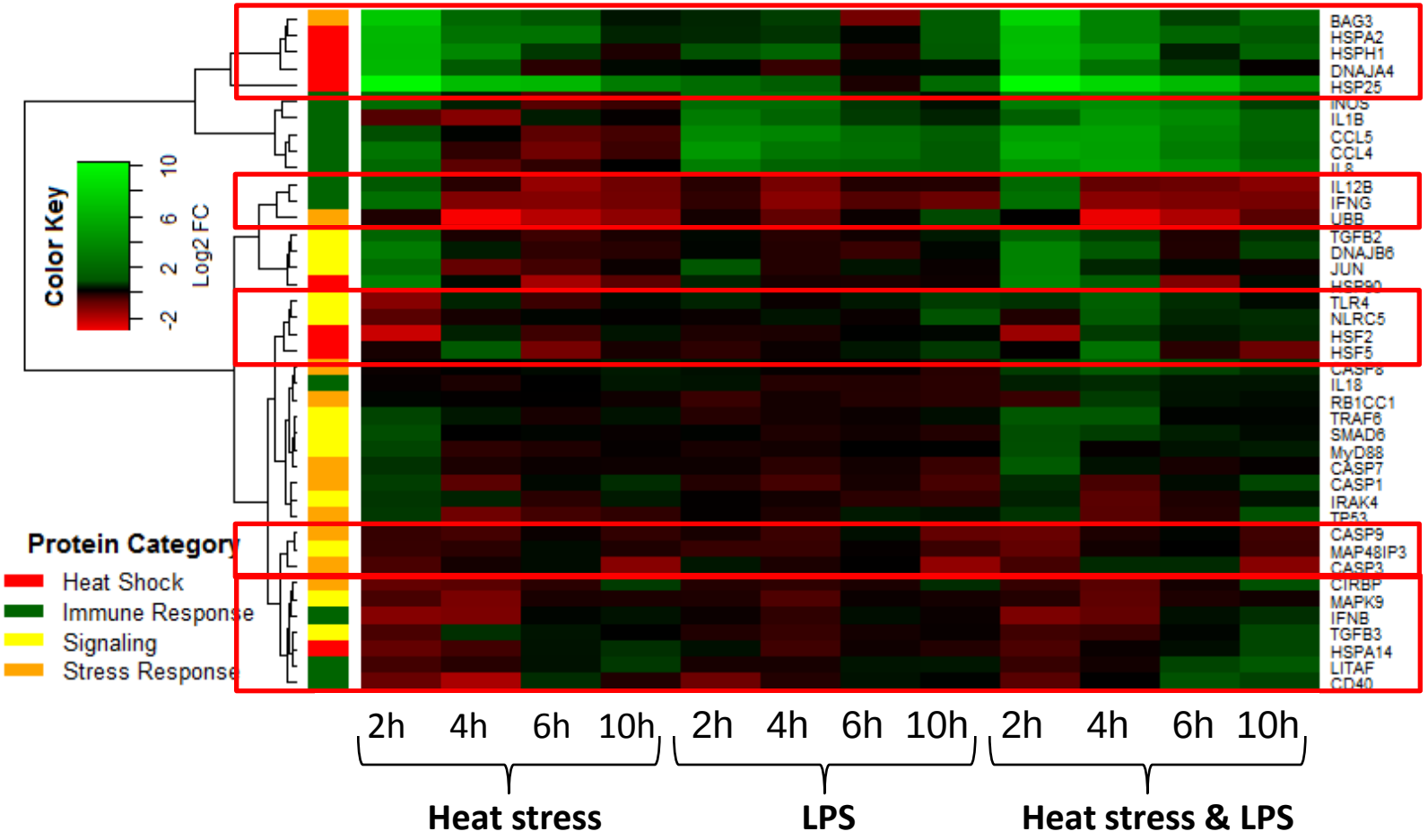
Signaling

MAPK9, MAPK8IP3, TGFB2, TGFB3, SMAD6, NLRC5, TLR4, MyD88, TRAF6, JUN, IRAK4

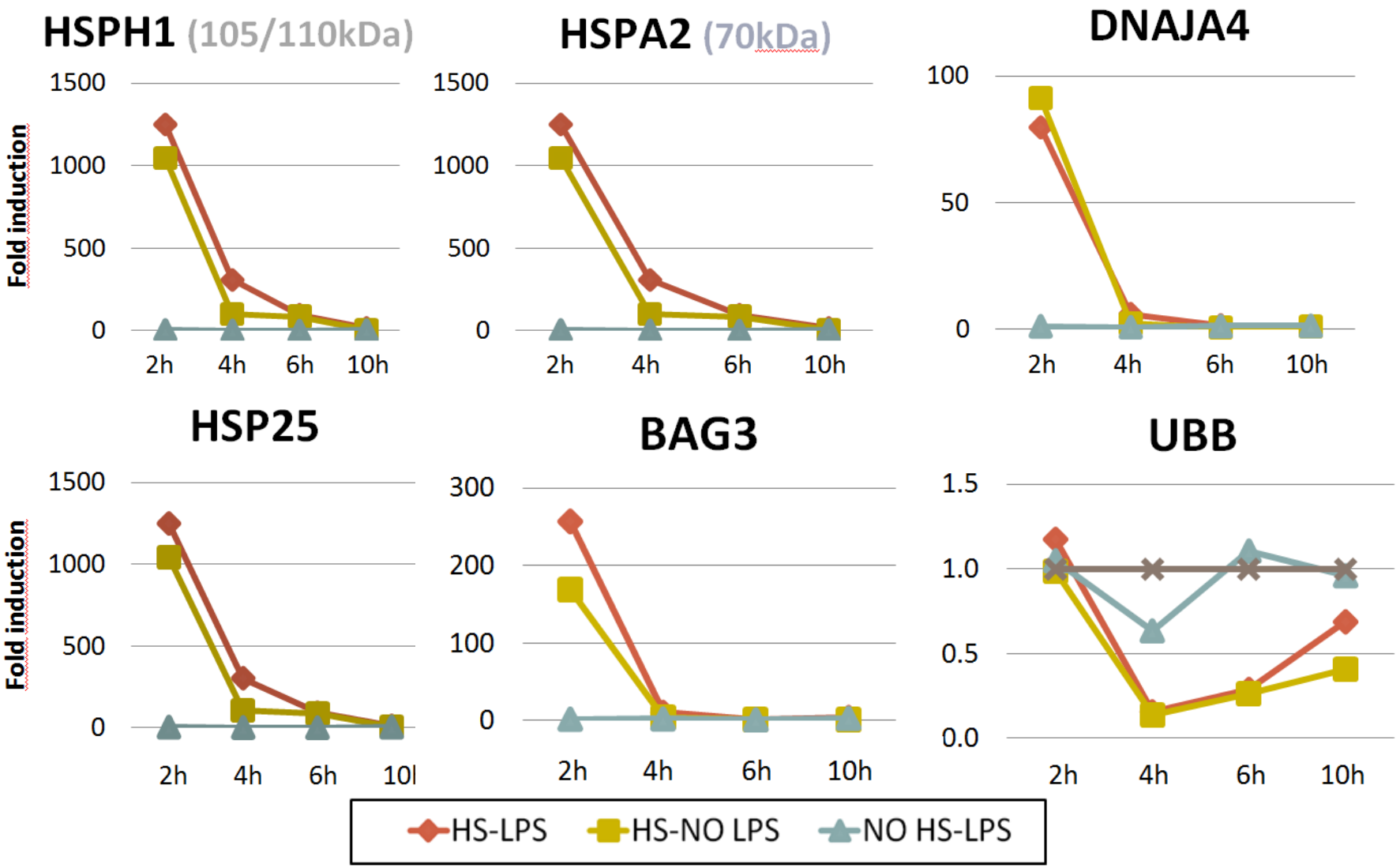
44 target genes + 2 housekeeping genes

FLUIDIGM

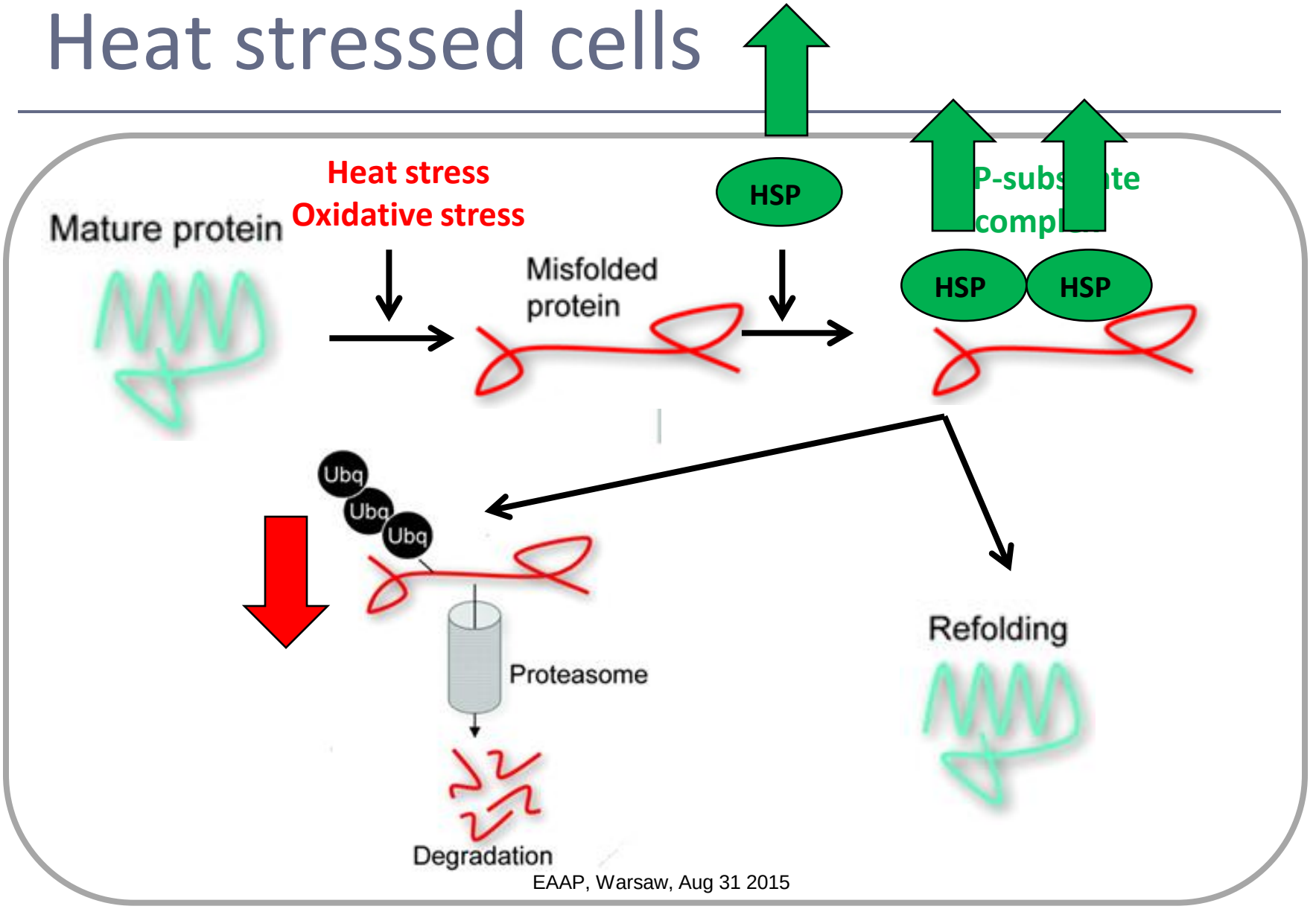
Log_2 Fold Change Cluster Analysis



Heat shock proteins, stress response

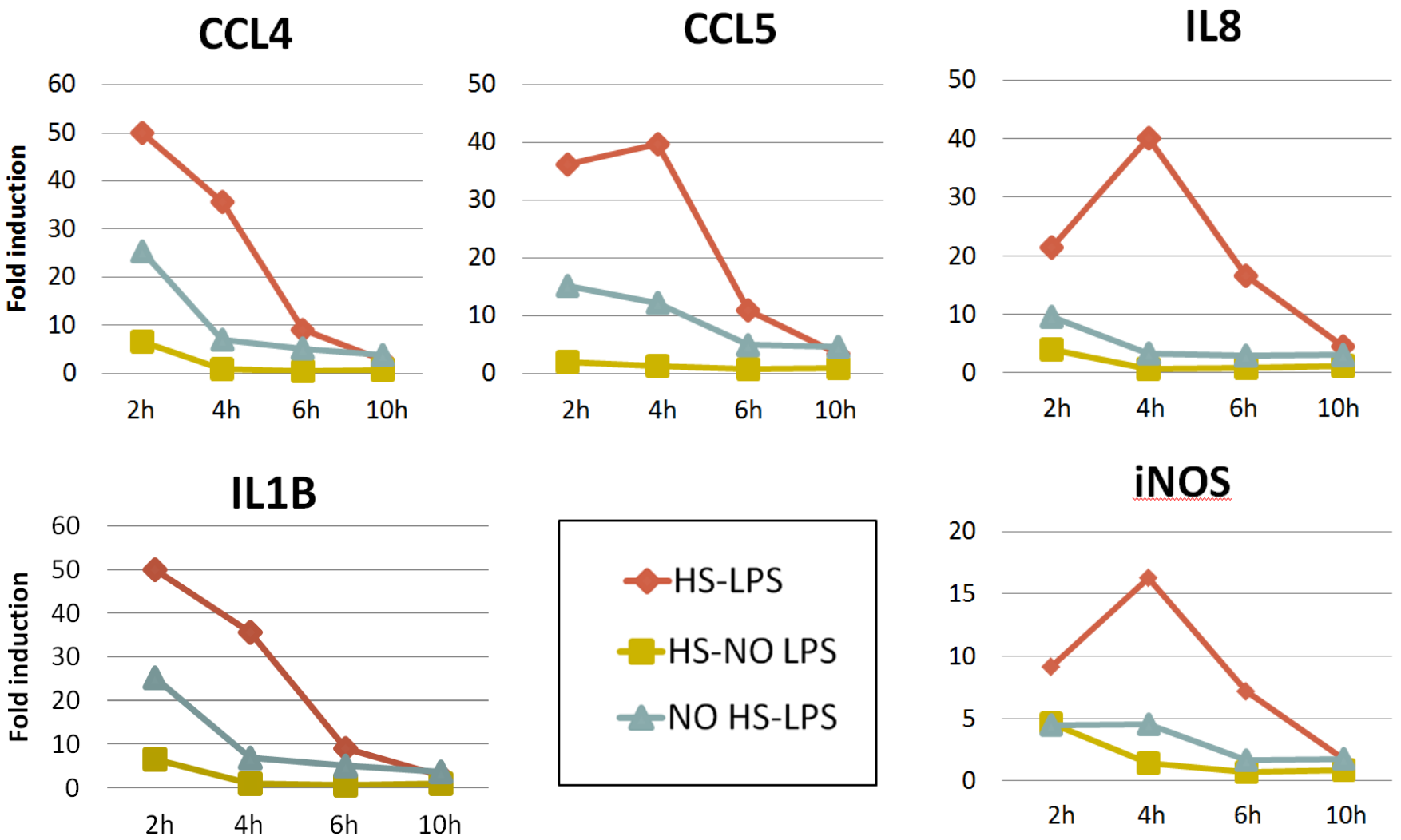


Heat stressed cells



Adapted from Benarroch (2011)

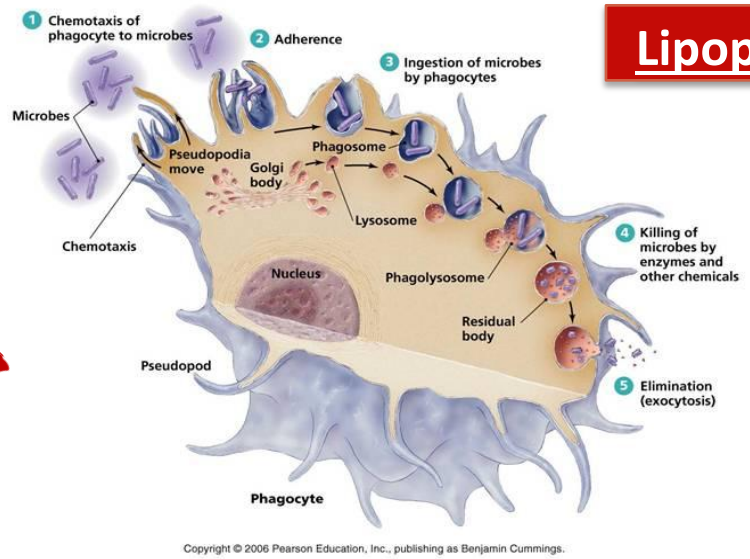
Immune-related genes



Immune response to LPS was reinforced by heat stress

HEAT STRESS

Lipopolisaccharide (LPS)



DAMP

Danger-associated molecular pattern

PAMP

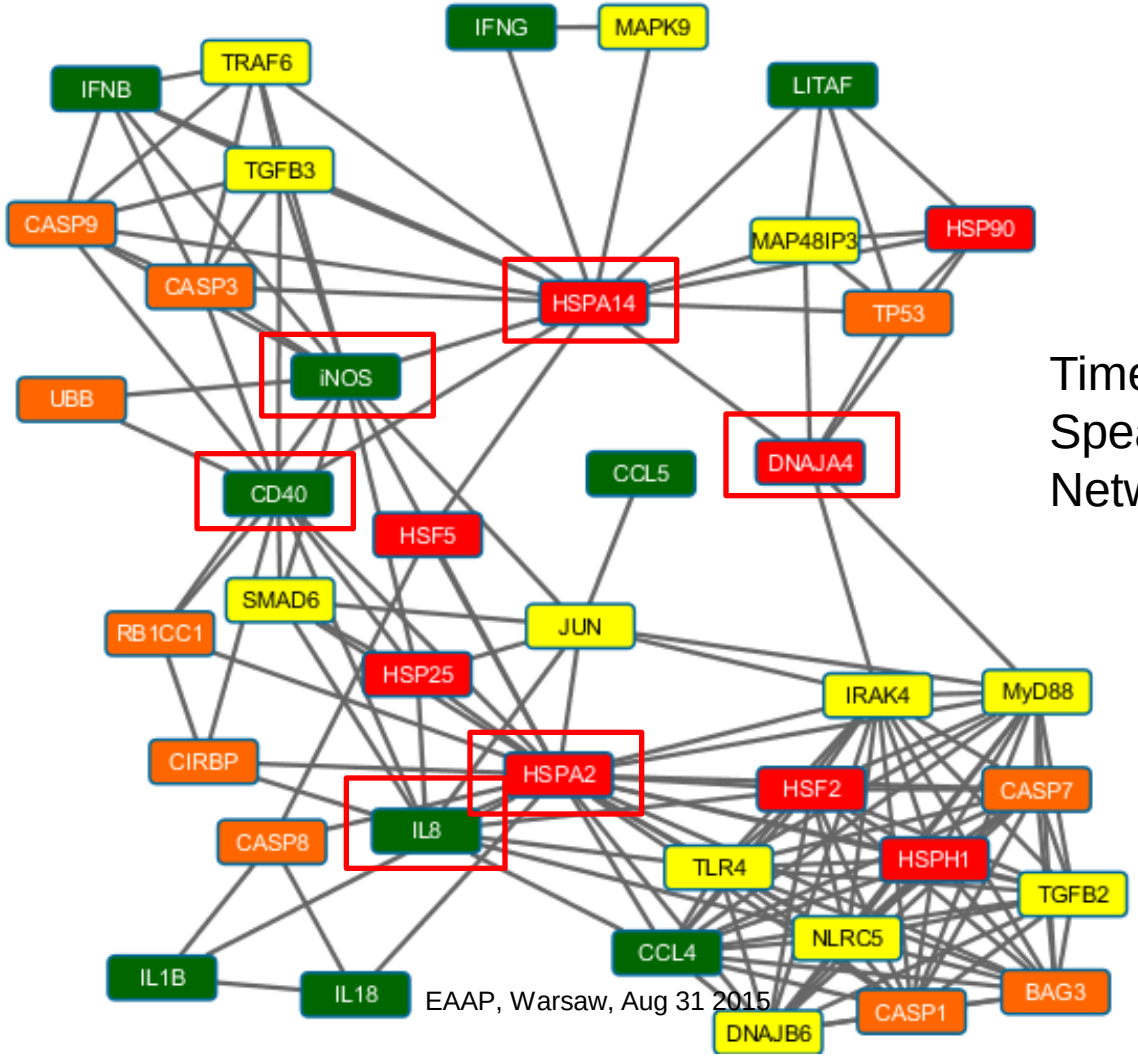
Pathogen-associated molecular pattern

Chemokines

Inflammation

Nitric Oxide

Spearman Correlation Network: Heat Stress

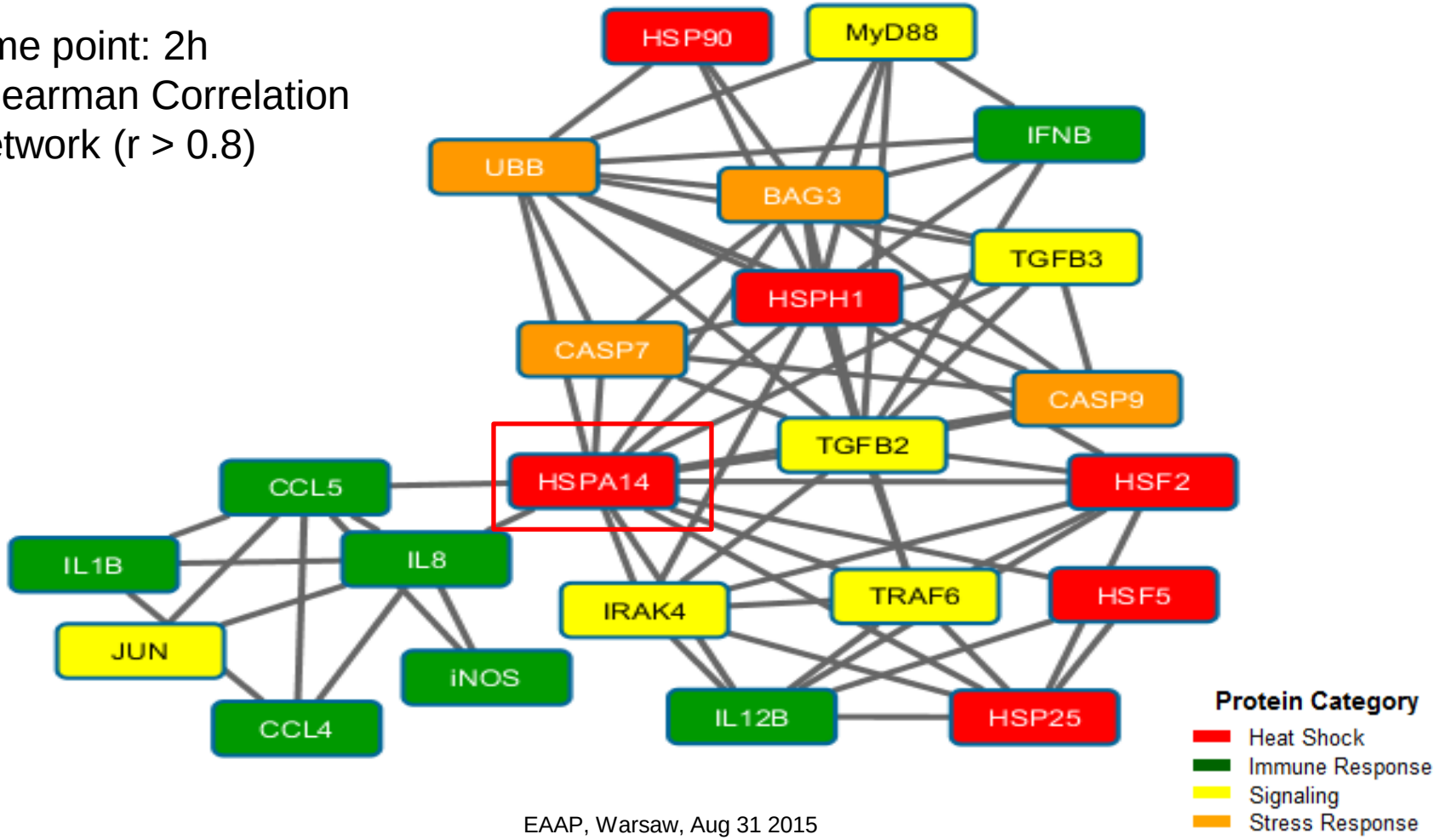


Time point: 2h
Spearman Correlation Network (r > 0.8)

Protein Category
- Heat Shock
- Immune Response
- Signaling
- Stress Response

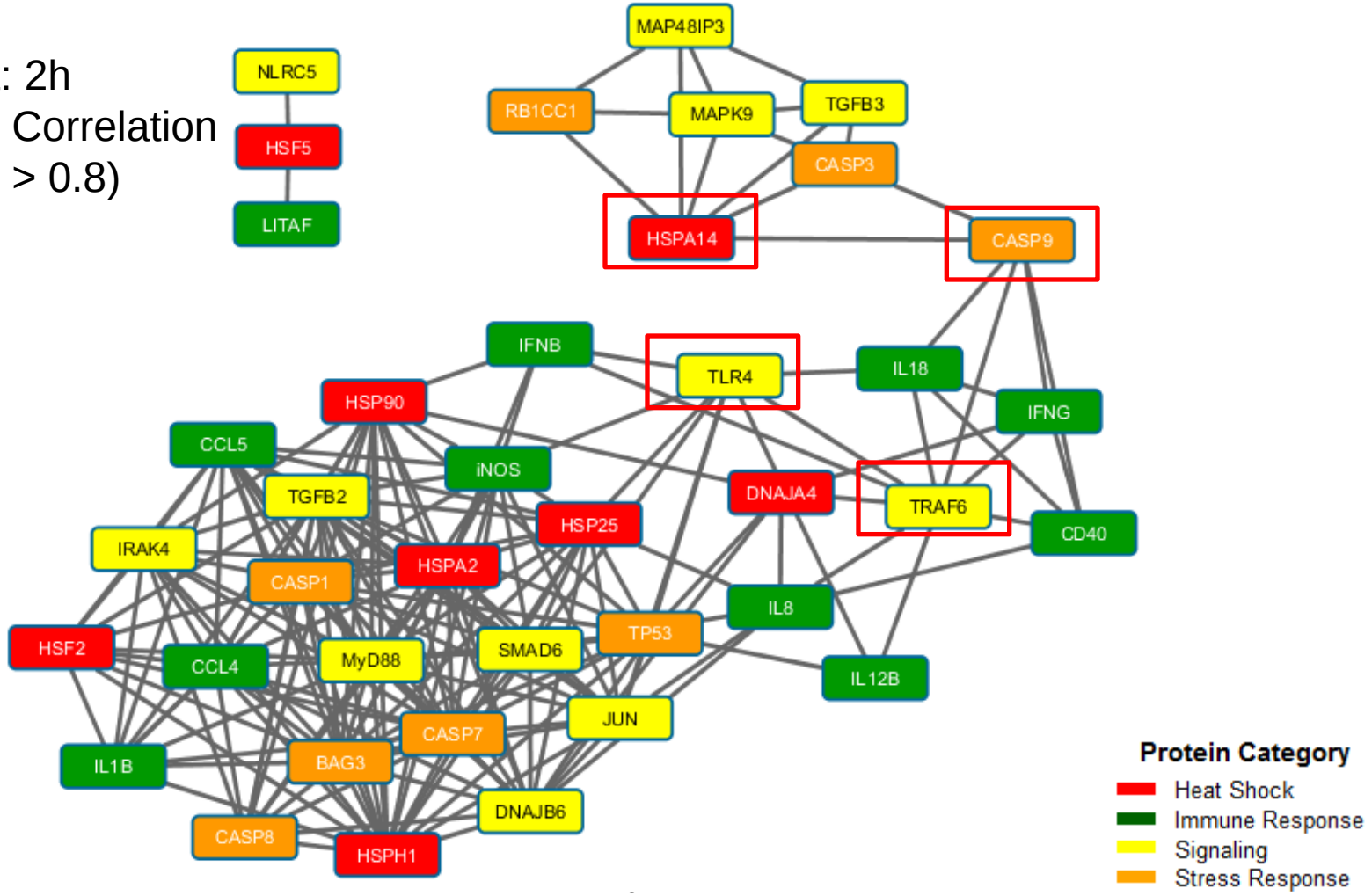
Spearman Correlation Network: LPS

Time point: 2h
Spearman Correlation Network (r > 0.8)



Spearman Correlation Network: Heat Stress & LPS

Time point: 2h
Spearman Correlation Network (r > 0.8)



Conclusions

1. The chaperones responsible for thermoregulation of the macrophages are: HSPH1, HSPA2 and HSP25
2. HSPA14 seems to have regulatory role in the gene network
3. Heat stress reinforced expression of LPS-activated chemokines: CCL4, CCL5, IL8, pro-inflammatory IL1B and inducible nitric oxide synthase (iNOS)
4. Synergistic effects of HS&LPS indicate the molecular response to the Danger-Associated Molecular Pattern (DAMP) provided by heat stress
5. There was similar regulation of the genes upon heat stress *in vivo* (literature) and *in vitro* (this study)

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Thank you!!!