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## Proteomic analysis of the Radiation-induced Bystander Effects between low-dose irradiated Chondrosarcoma cells and Bystander Chondrocytes

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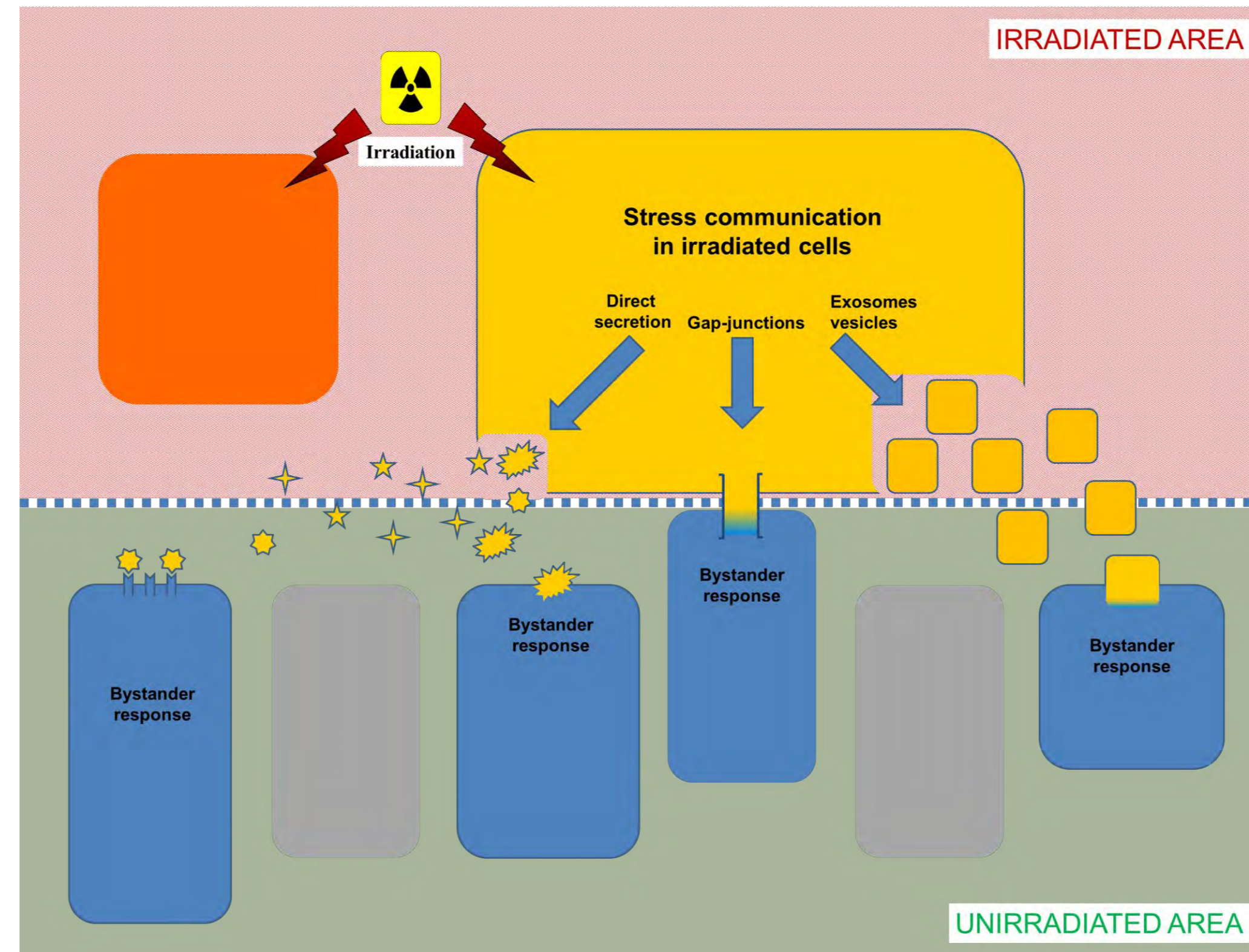
1 LARIA, CEA-DRF-IRCM, François JACOB Institute, Caen, France ; 2 CIMAP UMR6252, Normandie Univ, ENSICAEN, UNICAEN, CEA, CNRS, Caen, France  
3 BPMP, Mass Spectrometry Proteomics Platform, Montpellier, France ; 4 Innovative technologies for Detection and Diagnostics, CEA-DRF-Li2D, Marcoule, France  
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Experimental evidences show that, in addition to direct and targeted effects of radiations, another effect is observed within the surrounding un-irradiated area; irradiated cells relay a stress signal in this close proximity, the bystander effect. Neighbouring un-irradiated cells react to this bystander effect with a specific response, characteristic of a biological context, but with a close relationship to the biological response typically associated with direct radiation exposure. Bystander responses and bystander factors secreted by irradiated cells were observed and studied in many physical and biological conditions, in vitro and in vivo [1].

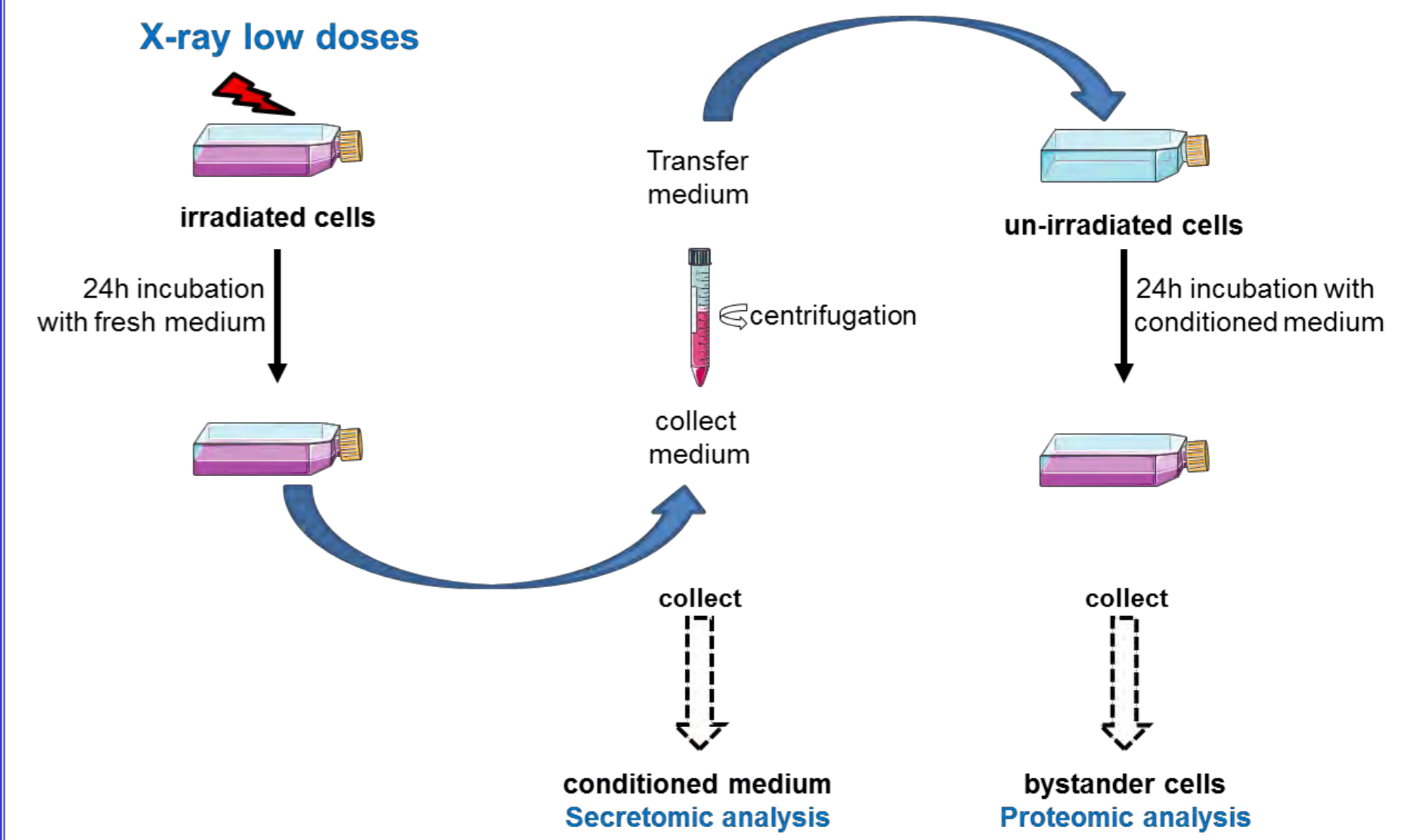
In the present study, we investigated the capacity of chondrocytes in responding to bystander factors released by irradiated chondrosarcoma cells using a medium transfer protocol. The cells were irradiated with low doses X-rays / Carbon ions and the bystander supernatant was transferred on non-irradiated cells. We already carried out survival and proliferation assays to study the effects of this treatment on the bystander cells [2].

In order to study the impact of these treatments on the cellular proteome, we carried out proteomic analysis starting from the cellular protein lysates and the conditioned medium. The proteomic analysis showed the effect of irradiation on the secretome of chondrosarcoma cells as well as the bystander effect on the proteome of bystander chondrocytes. We then selected variant spots that were analysed by mass spectrometry. An in-depth analysis of the identified proteins will provide a better understanding of the mechanisms involved in cell irradiation as well as the radio-induced Bystander effect.

1. Chevalier F, et al. Proteomic overview and perspectives of the radiation-induced bystander effects. Mutation Research/Reviews in Mutation Research. 2014;763:280-93.  
2. Lepleux C, et al. Bystander effects of chondrosarcoma cells irradiated at different LET impart proliferation of chondrocytes. J Cell Commun Signal. 2015.



### Experimental strategy



### Set up experiments

#### Irradiation conditions

- X-rays
- low doses
- 0,1 Gy vs 0 Gy

#### Biological model

- chondrosarcoma (SW1353)
- chondrocytes (T/C-28A2)

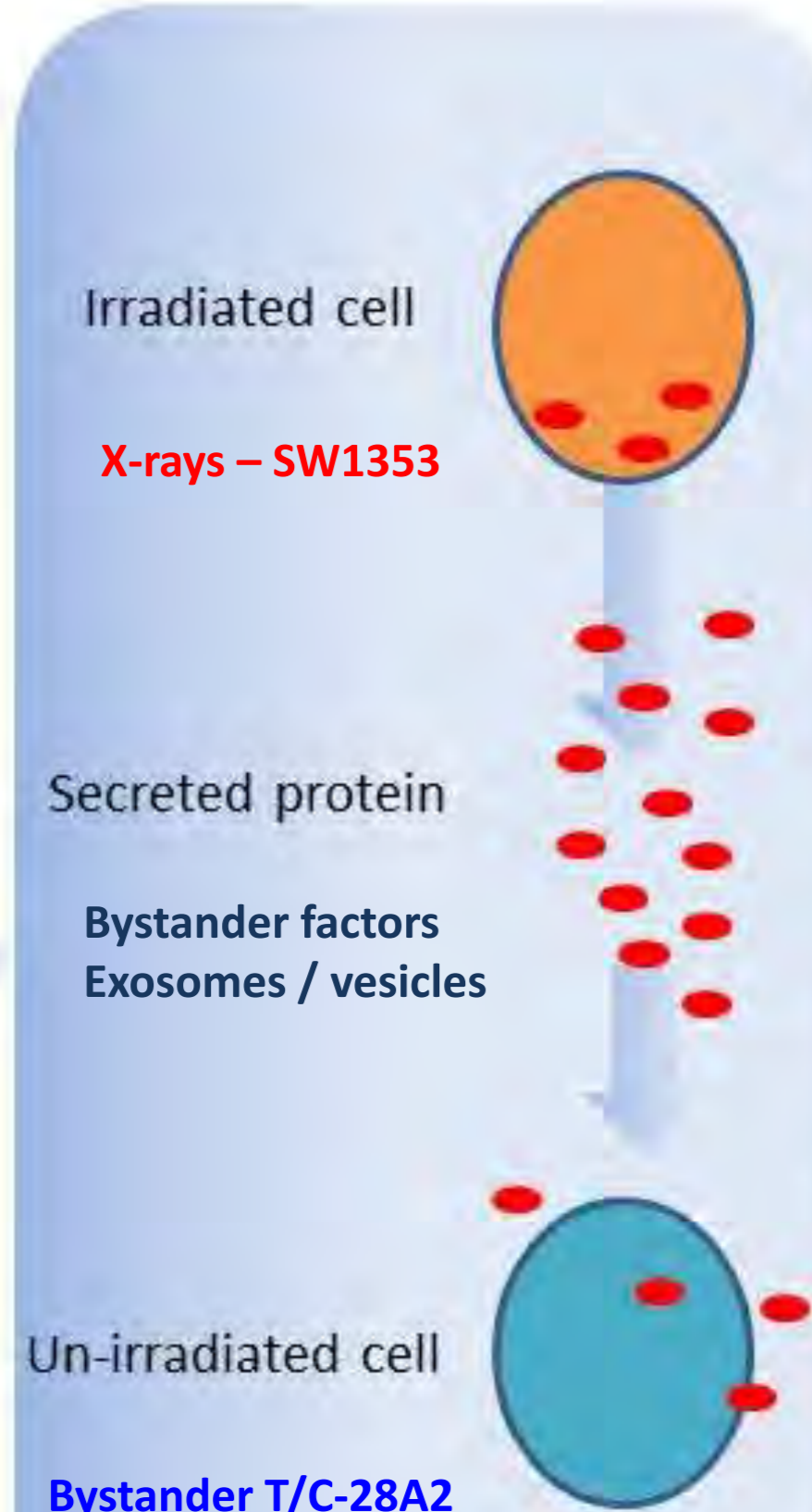
#### Bystander mechanism studied

- Secretion of factors
- Secretion of vesicles

#### Analysis protocol

- Medium transfer
- Proteomic analysis
- Proteome
- secretome

### Bystander area



### Proteomic domain

#### Proteome analysis of direct effects

#### Secretome analysis

#### Proteome analysis of indirect effects

### Exosomes and secreted proteins : potential bystander factors

Conditioned medium (SW1353 0,1Gy) vs conditioned medium (SW1353 0Gy)

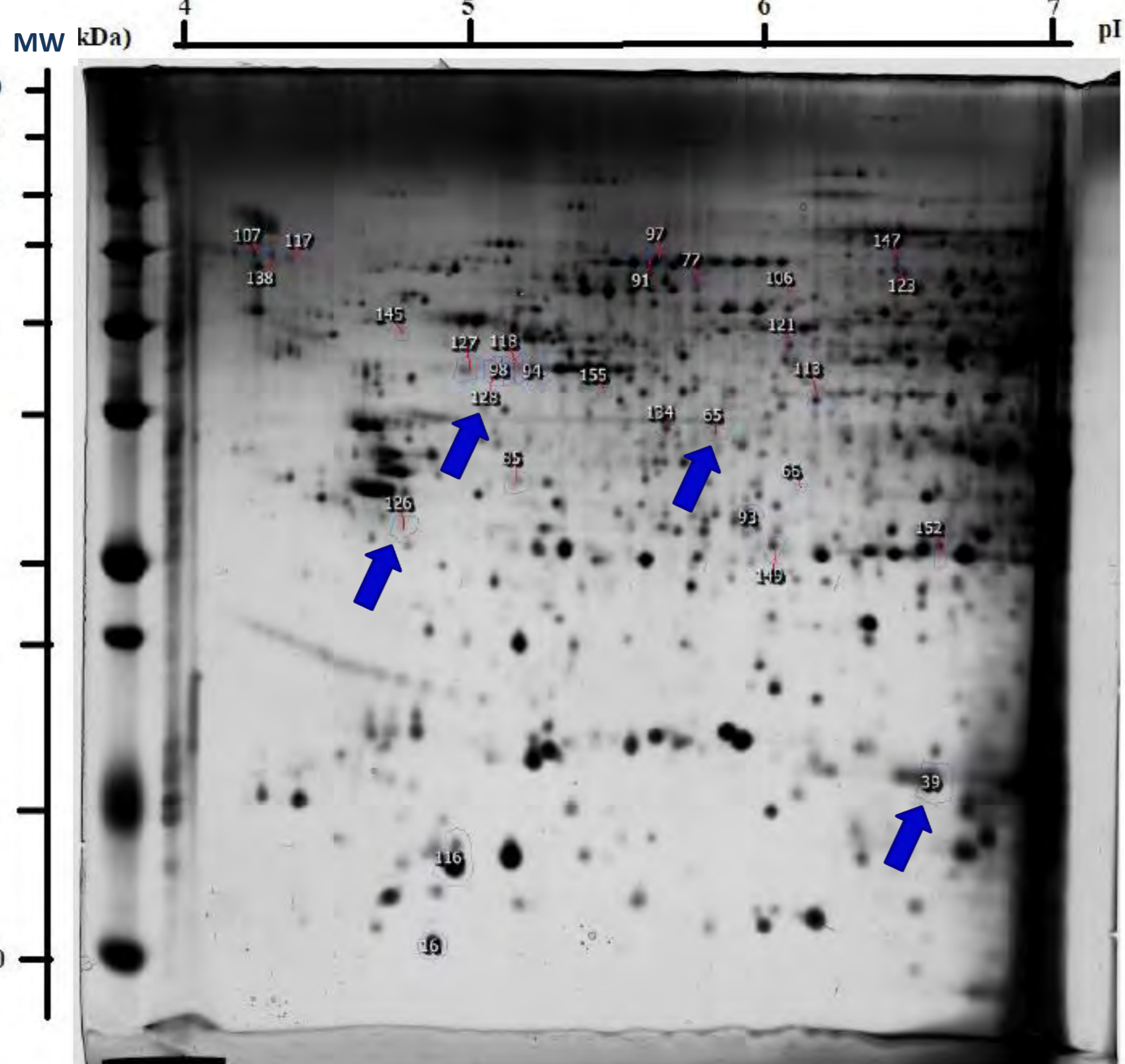
- From the 1698 proteins identified, 66 were differentially expressed in conditioned media (42 ↑ and 24 ↓)
- examples of secreted proteins:

accession	description	Fold
O43790-04	Keratin, type II cuticular Hb6	7,67
P23528	Cofilin-1	4,85
Q13257	Mitotic spindle assembly checkpoint protein MAD2A	2,83
P40227-01	T-complex protein 1 subunit zeta	2,81
P38159	RNA-binding motif protein, X chromosome	2,31
P42167-01	Lamina-associated polypeptide 2, isoforms beta/gamma	2,27
Q14847	LIM and SH3 domain protein 1	2,11
P61163	Alpha-centractin	2,00
Q00325-01	Phosphate carrier protein, mitochondrial	2,00
A6NDG6	Glycerol-3-phosphate phosphatase	1,88
O00232-01	26S proteasome non-ATPase regulatory subunit 12	1,80
P30566-01	Adenylosuccinate lyase	1,70
P30405	Peptidyl-prolyl cis-trans isomerase F, mitochondrial	1,58
Q99798-01	Aconitate hydratase, mitochondrial	1,54
P61769-01	Beta-2-microglobulin	1,50
Q9NZN4-01	EH domain-containing protein 2	1,50
PS4577-01	Tyrosine-tRNA ligase, cytoplasmic	-1,64
Q9UBM7-01	7-dehydrocholesterol reductase	-1,67
Q14152-03	Eukaryotic translation initiation factor 3 subunit A	-1,80
P13861	cAMP dependent protein kinase type II-alpha regulatory subunit	-2,00
P49189-01	4-trimethylaminobutyraldehyde dehydrogenase	-2,67

- TNFR1 exosome secretion
- Actin regulation, ion binding and carrying
- Mitochondrial permeability
- glucose metabolism
- glucose metabolism
- Mitochondrial permeability
- MAPK pathway regulation
- Mitochondrial permeability

Protein extraction and separation: Proteins were TCA precipitated and then solubilized in laemmli buffer, and separated by SDS-PAGE.  
Mass spectrometry: Each bands from the SDS-PAGE gels were picked and analysed by LC-MS/MS (Q-EX) mass spectrometry

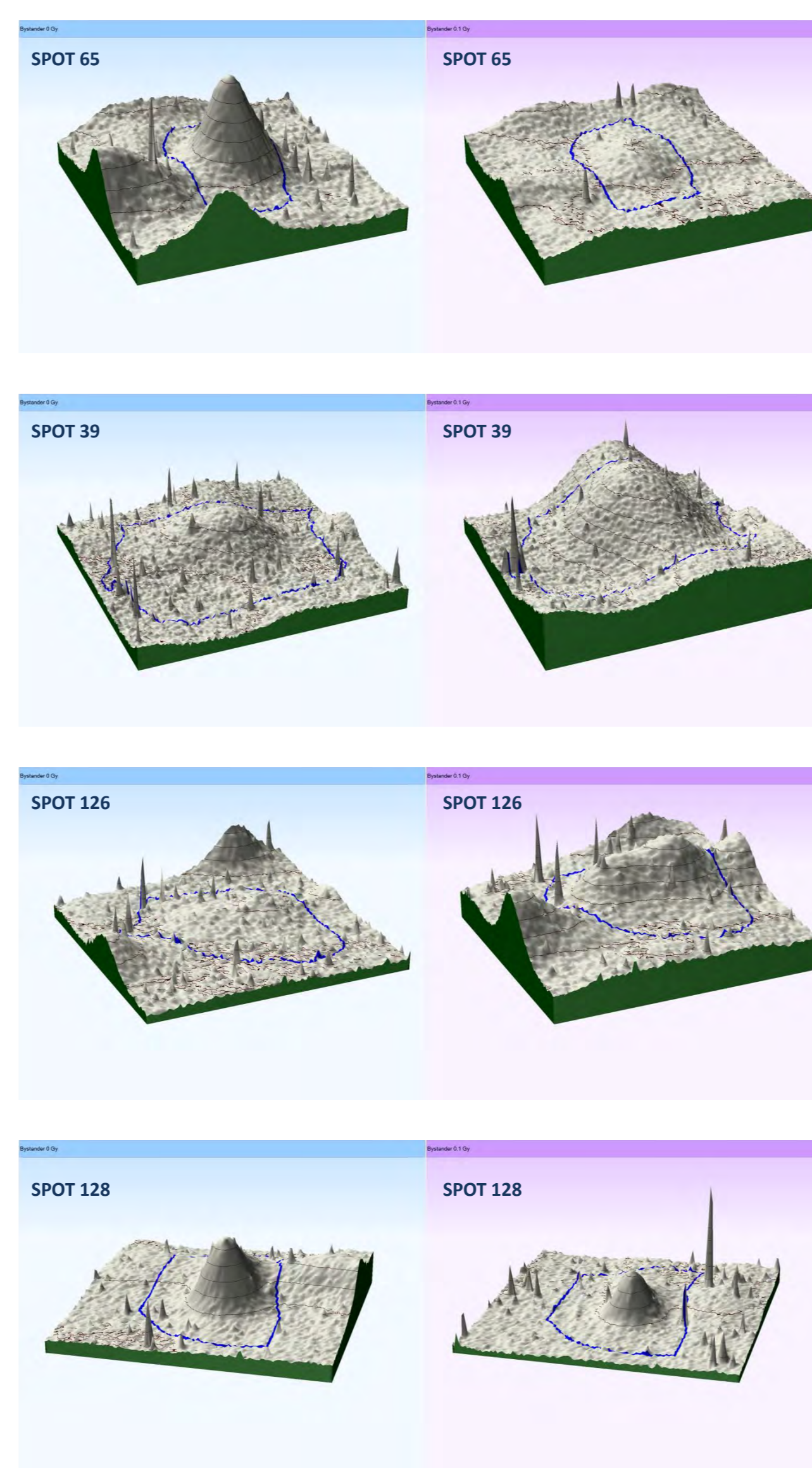
### Modulated proteins in bystander cells : potential bystander responses Bystander T/C-28A2 (0,1Gy) vs control T/C-28A2 (0Gy)



**Protein extraction and solubilisation**  
Proteins were extracted from TC-28/AC cells in sample buffer containing 7 M urea, 2M thiourea, 4% CHAPS, 0.05% Triton X100, 65 mM DTT, 40mM spermine, protease and phosphatase inhibitor cocktails. Proteins were then precipitated with the 2D clean-up kit (GE Healthcare) and the pellet was solubilized with TUC solution (7M urea, 2M thiourea, 4% CHAPS) and quantified with the 2D quant kit (GE Healthcare).

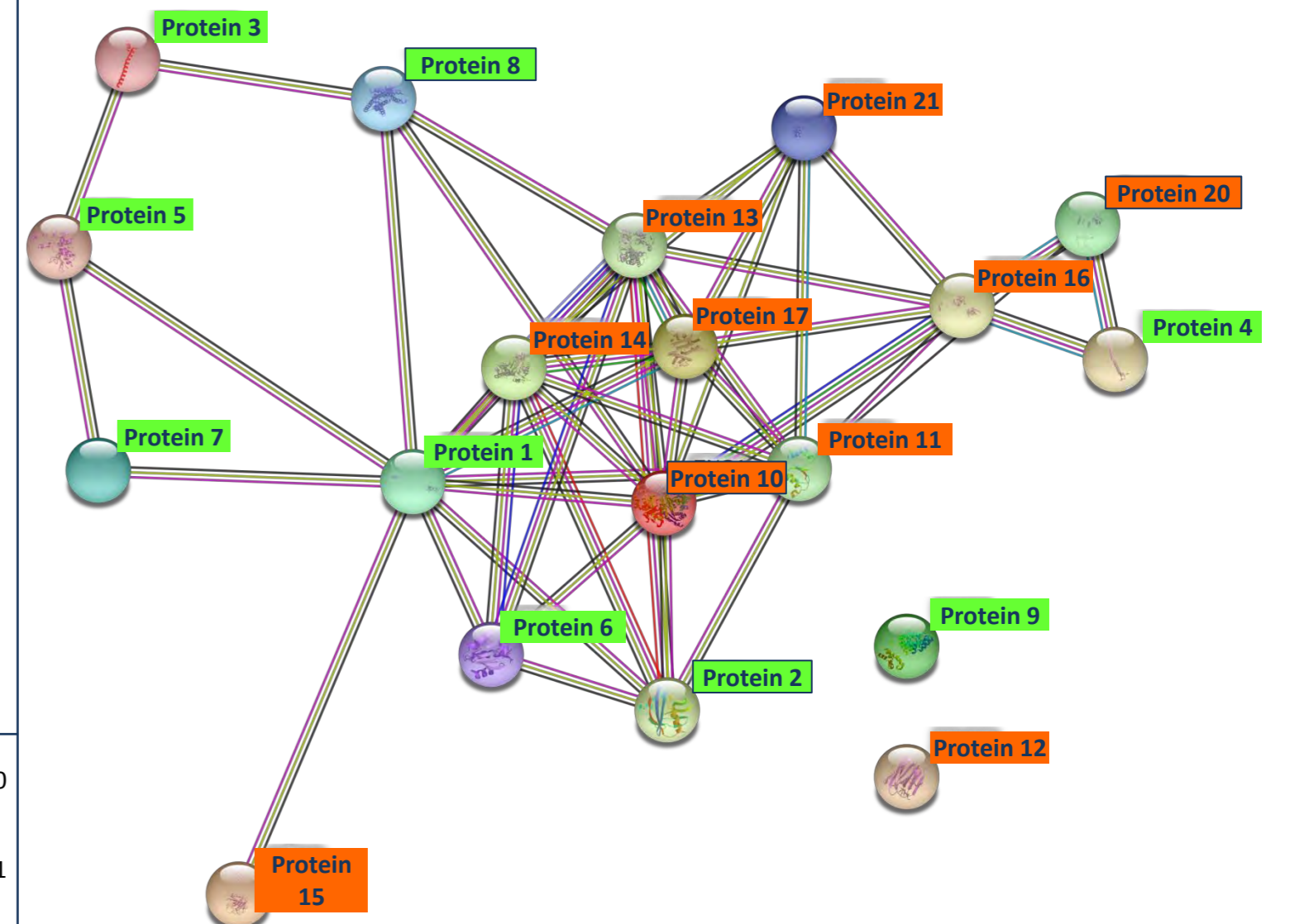
**Protein load : 250 µg and 2D electrophoresis (6 technical / biological replicates)**  
IPG strips : pI 4-7, 18 cm ; 1<sup>st</sup> dimension - Protean IEF Cell (Bio-Rad)  
2<sup>nd</sup> dimension : 12% acrylamide gel 20cm x 20cm (dodeca Cell electrophoresis unit, Bio-Rad)  
Gel staining and picture acquisition : Silver stain, stained gels scanned on a GS 800 densitometer (Bio-Rad)

**2D gel analysis and Mass spectrometry**  
Stained gels were analysed with the Progenesis SameSpots software and variant spots were picked and analysed by LC-MS/MS (Q-EX) mass spectrometry



Spots N°	Fold	pI	MW	Name
16	1,31	4,79	20	Protein 1
39	1,62	6,69	26	Protein 2
85	1,38	5,11	63	Protein 3
93	1,31	5,99	57	Protein 4
116	1,27	4,87	23	Protein 5
116.2	1,27	4,87	23	Protein 6
126	1,63	4,67	56	Protein 7
126.2	1,62	4,67	56	Protein 8
145	1,47	4,67	98	Protein 9
65	1,81	5,86	72	Protein 10
65.2	1,81	5,86	72	Protein 11
77	1,55	5,79	130	Protein 12
91	1,29	5,63	140	Protein 13
97	1,32	5,64	141	Protein 14
113	1,27	6,25	79	Protein 15
121	1,47	6,15	94	Protein 16
123	1,31	6,56	136	Protein 17
127	1,31	4,91	87	Protein 18
128	1,36	5,03	87	Protein 20
134	1,26	5,68	73	Protein 21

### String network of modulated accessions



green spot : increased in bystander ; red spot : decreased in bystander

- Protein 10:** Multifunctional enzyme that, as well as its role in glycolysis, plays a part in various processes such as growth control, hypoxia tolerance and allergic responses. Used as a diagnostic marker for many tumors, as a marker for hypoxic brain injury after cardiac arrest
- Protein 2:** accelerate the folding of proteins, involved in irradiation response and can be secreted in response to oxidative stress in vascular smooth muscle through a vesicular secretory pathway that involves actin remodeling and myosin II activation, and mediates ERK1/2 activation.
- Protein 8:** Negative regulator of signaling cascades that mediate activation of MAP kinases; Negative regulator of osteogenesis
- Protein 20:** plays a key role in the maintenance of protein homeostasis by removing misfolded or damaged proteins, which could impair cellular functions, and by removing proteins whose functions are no longer required.